

204040" 254T4660

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#6

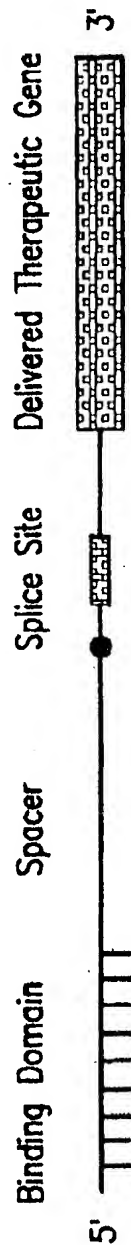


FIG.1A

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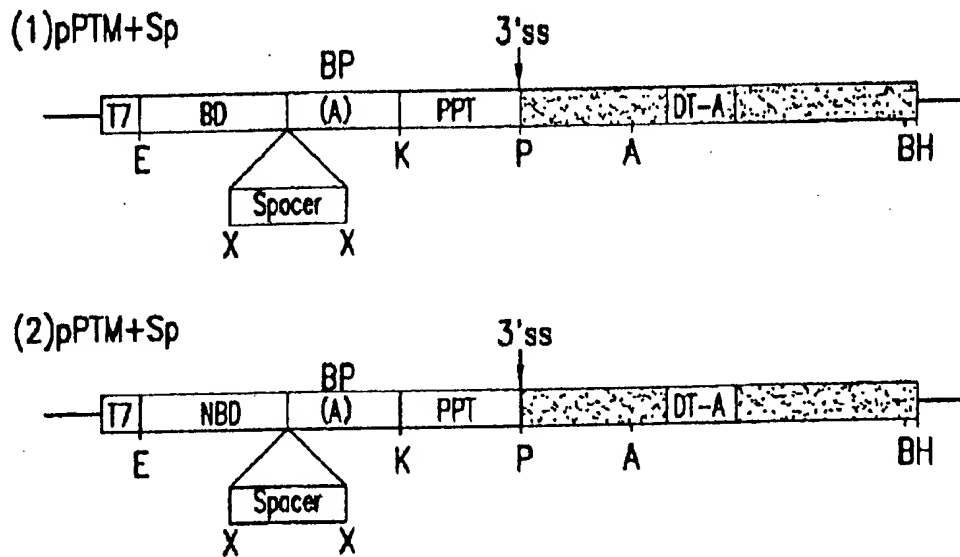


FIG.1B

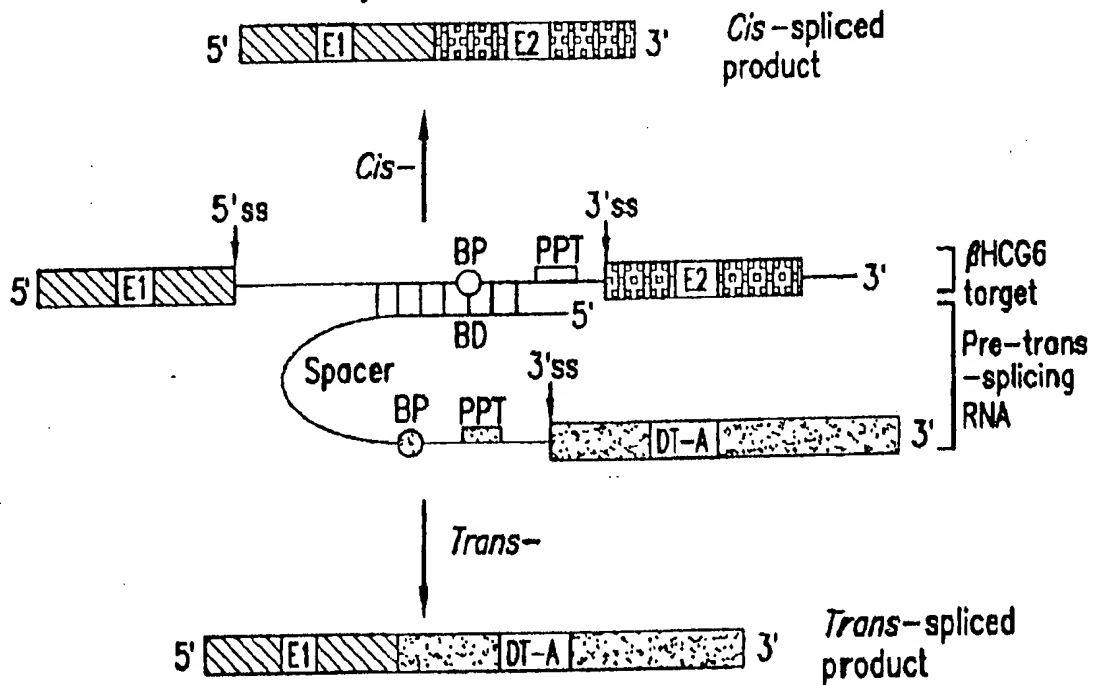


FIG.1C

204040-2544650

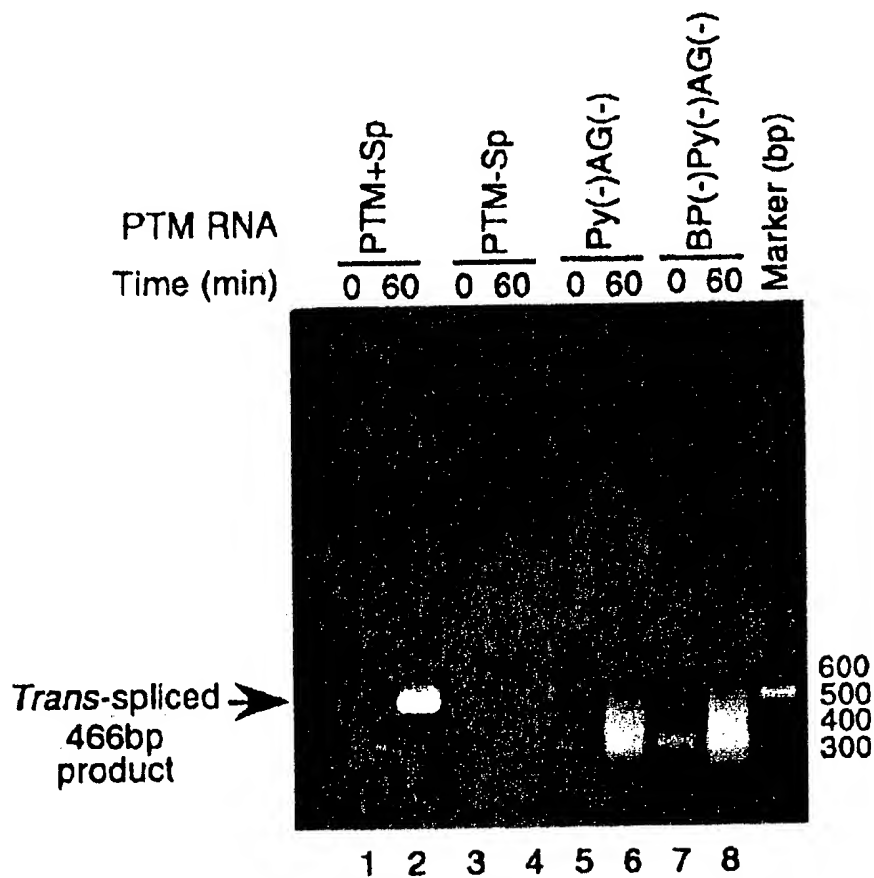


FIG.2A

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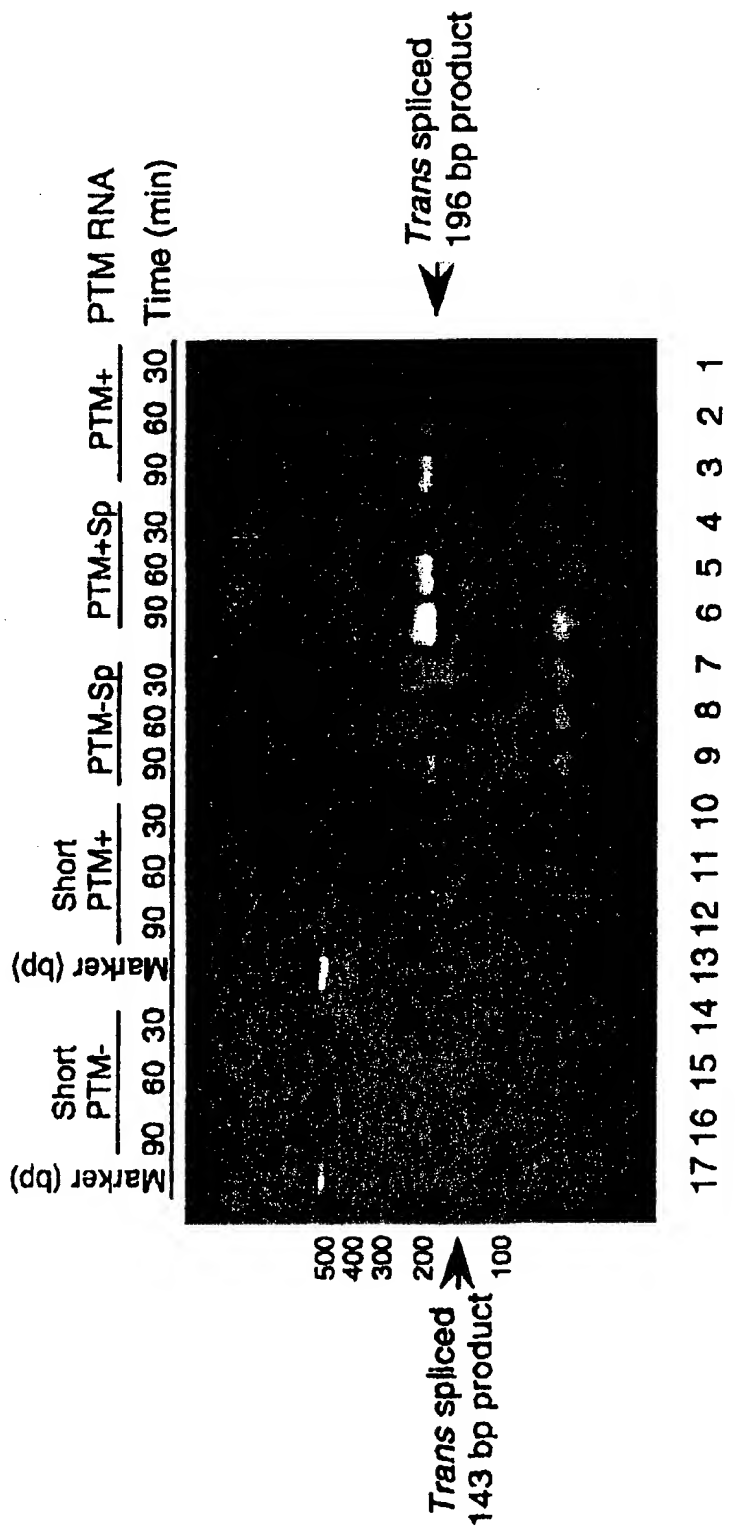


FIG.2B

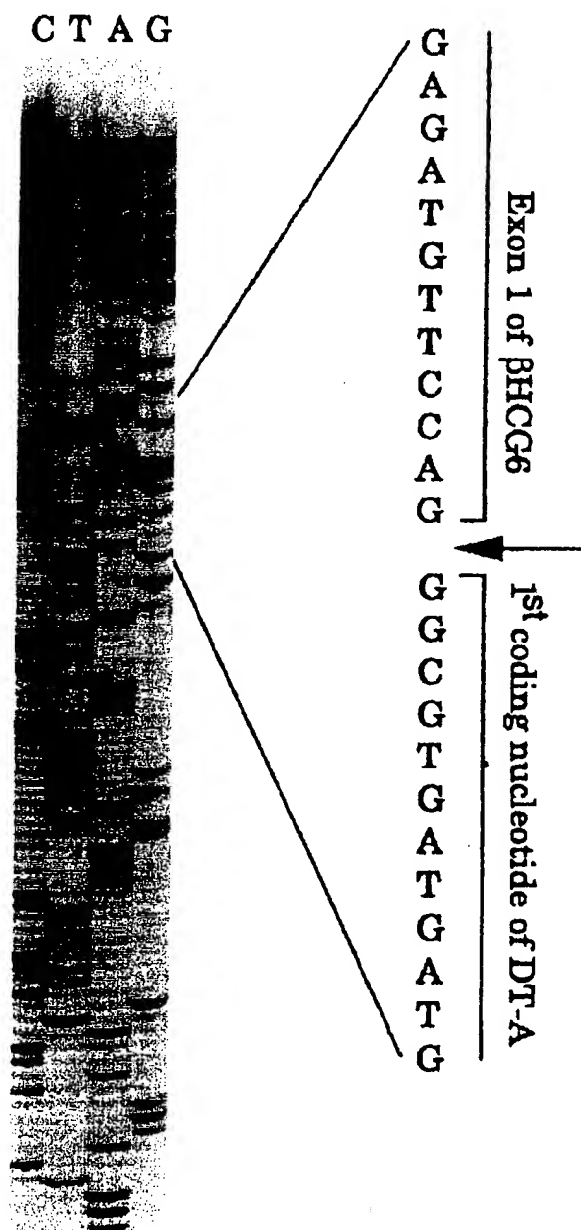
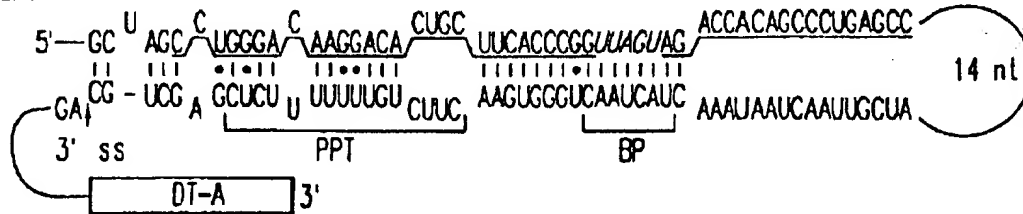


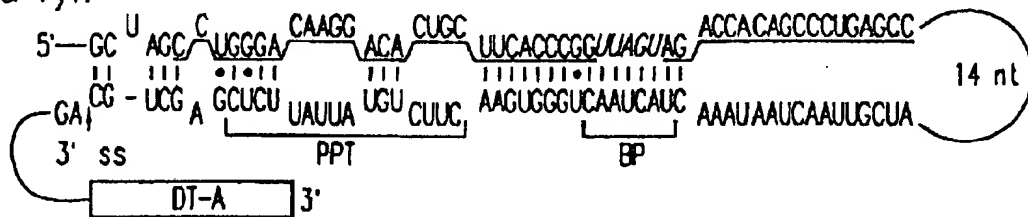
FIG.3

09941492.040402

1. PTM+SF:



2. PTM+SF-Py1:



3. PTM+SF-Py2:

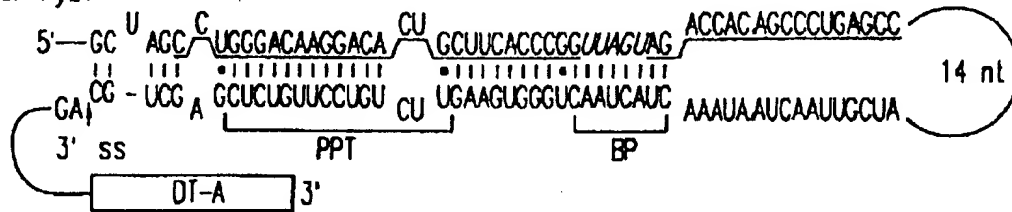


FIG.4A

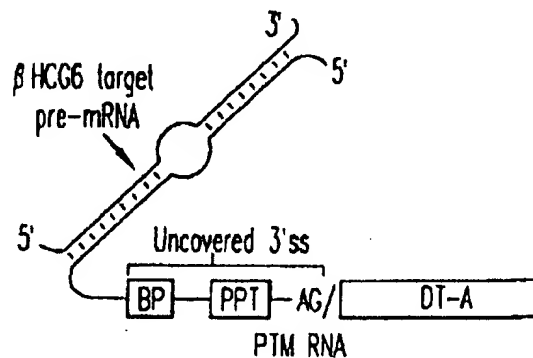


FIG.4B

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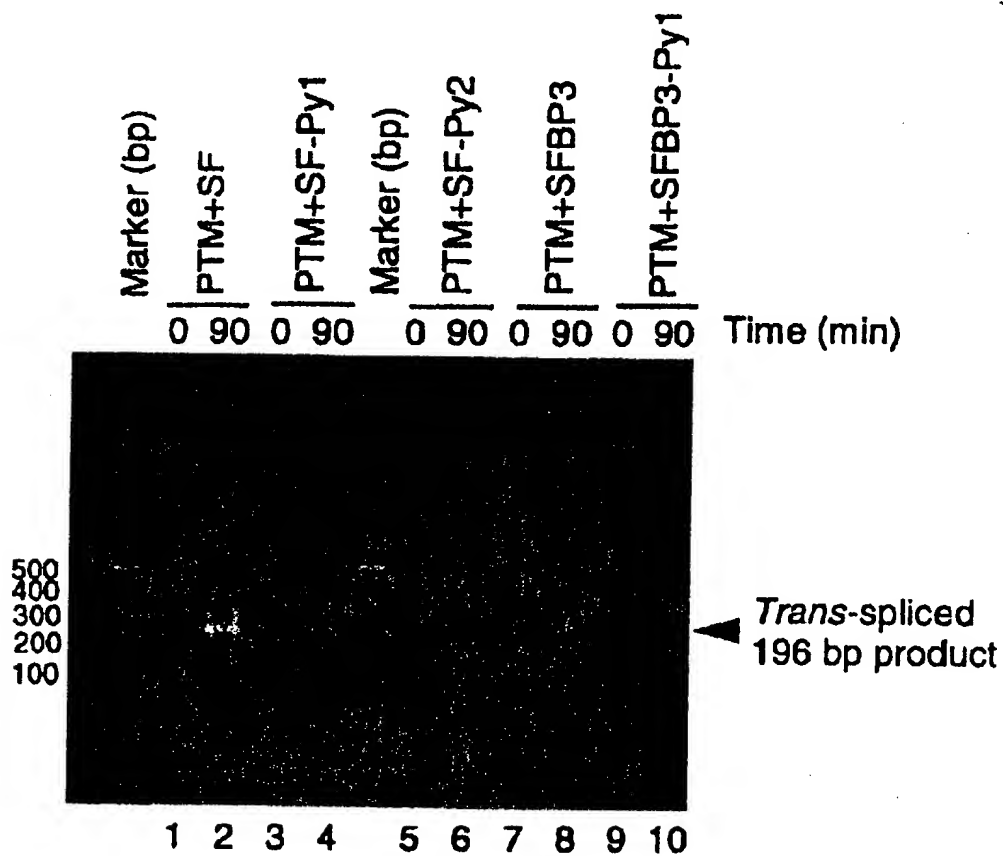


FIG.4C

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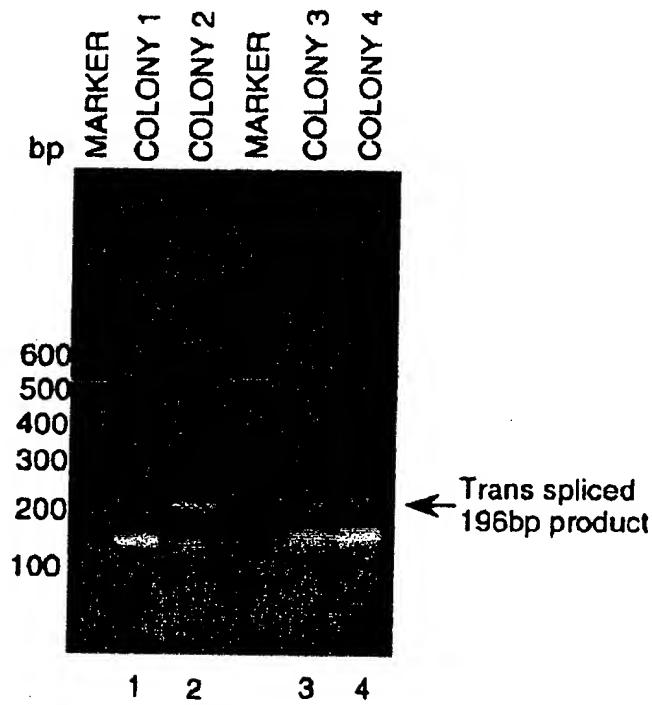


FIG.7A

204040" 2647450

EXON 1 OF β HCG6 †
5'-CAGGGACGCACCAAGGATGGAGATGTTCCAG-GGCGTGATGATGTTGTT
† 1ST CODING NUCLEOTIDE OF DT-A
GATTCTTCTTAAATCTTTTIGATGGAAACTTTTCTTCTGTTACCAACGGGACTA
AACCTGGTTAIGTAGATTCCATTCAAAA-3'

11 8 91

FIG.7B

12 8 91

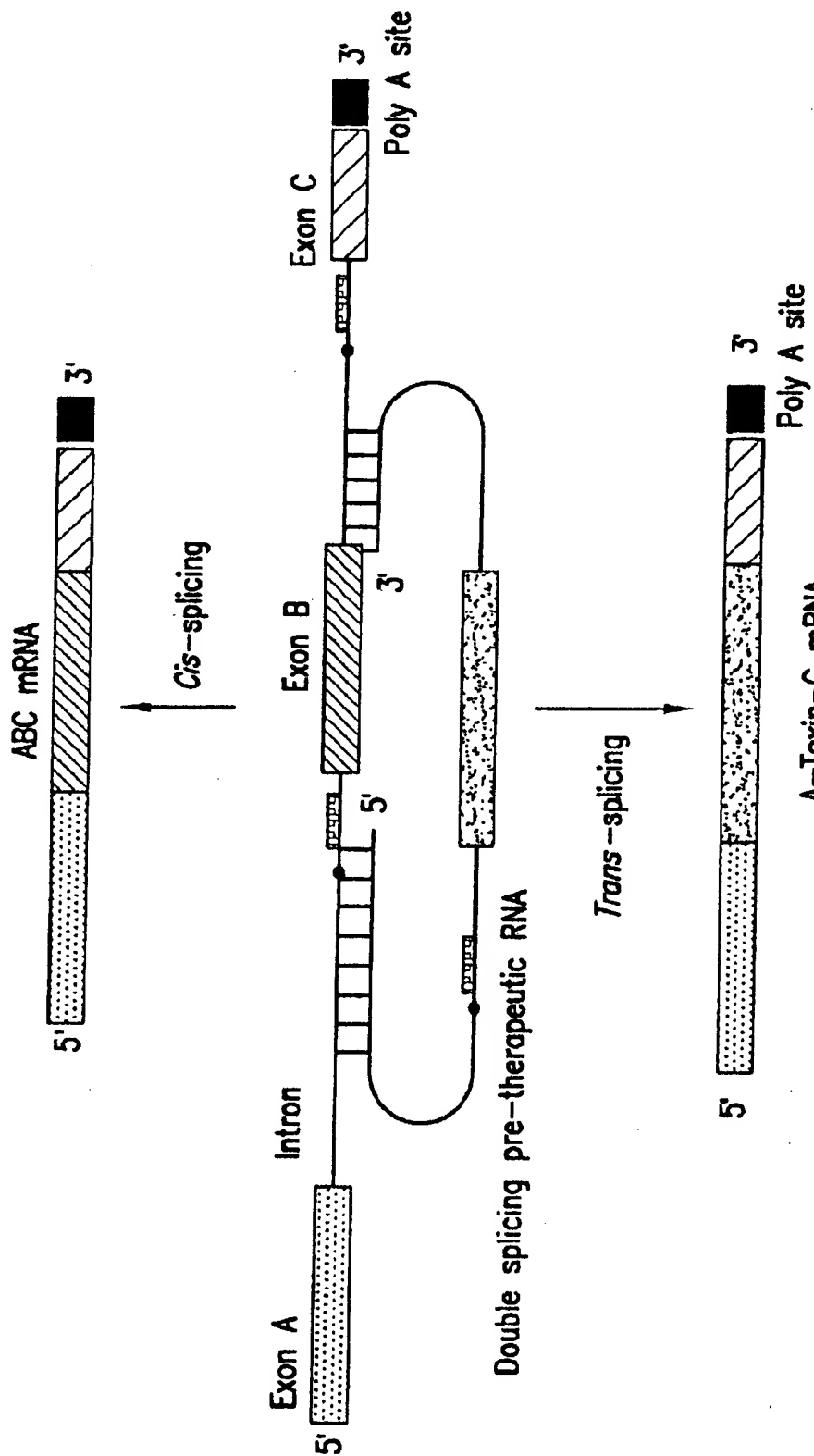
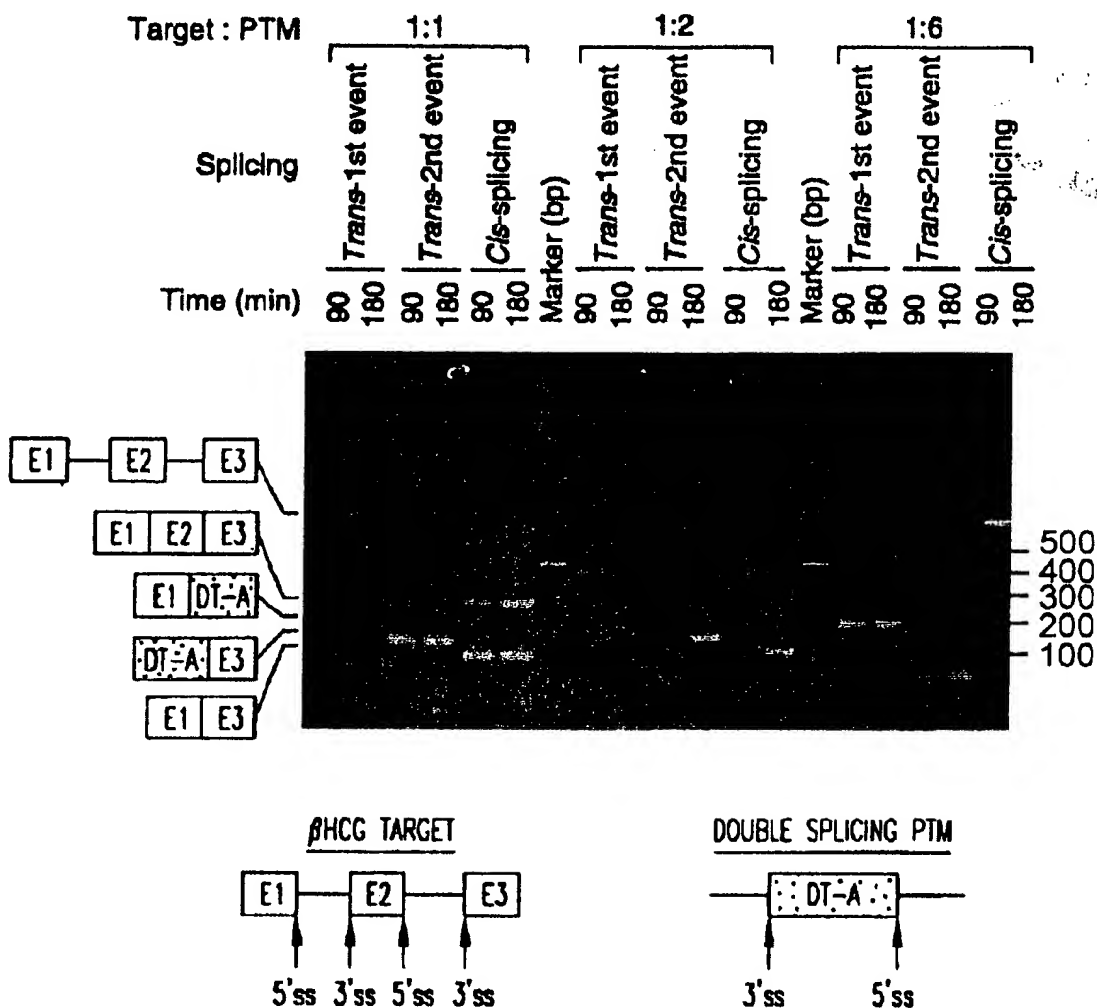


FIG.8A



Cis-spliced products

E1 E2 E3 = NORMAL cis-SPLICING (277bp)

E1 E3 = Exon SKIPPING (110bp)

Trans-spliced products

E1 DT-A = 1st EVENT, 196bp. Trans-SPLICING BETWEEN 5' ss OF TARGET & 3' ss OF PTM.

DT-A E3 = 2nd EVENT, 161bp. Trans-SPLICING BETWEEN 3' ss OF TARGET & 5' ss OF PTM.

FIG.8B

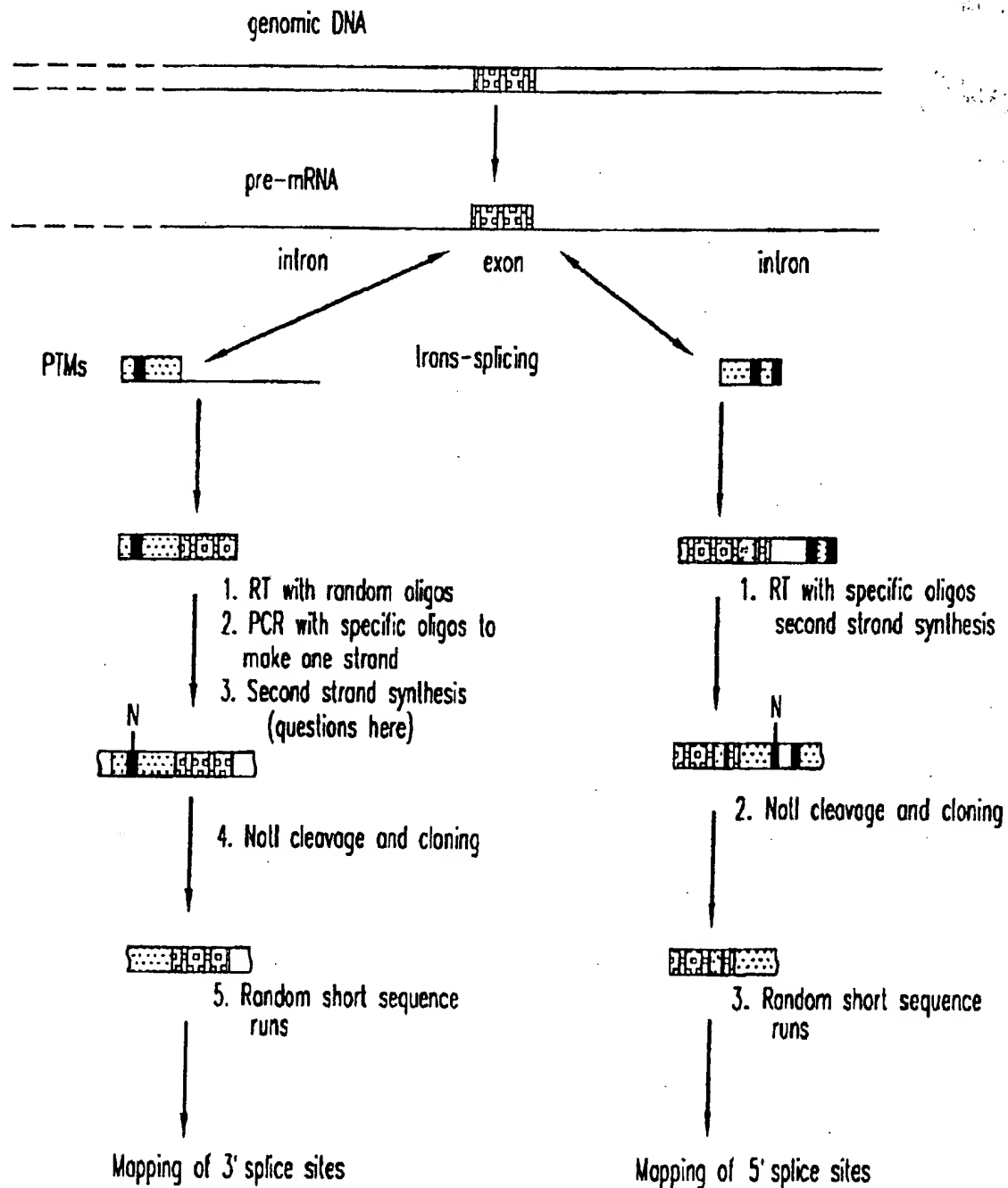


FIG.9

2014040"26171650

17 8 91

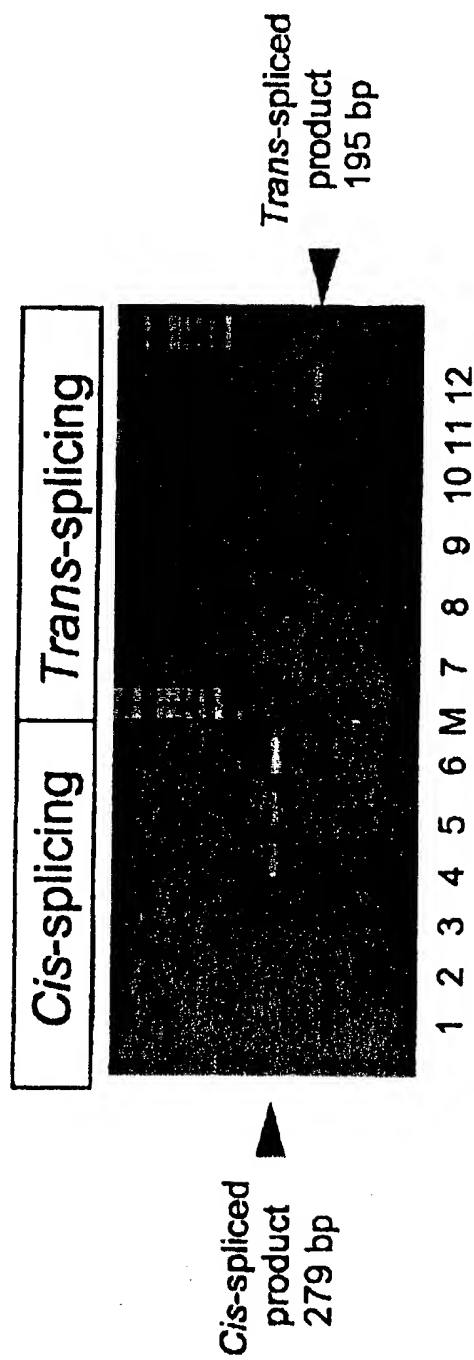


FIG.11A

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204040 25474650

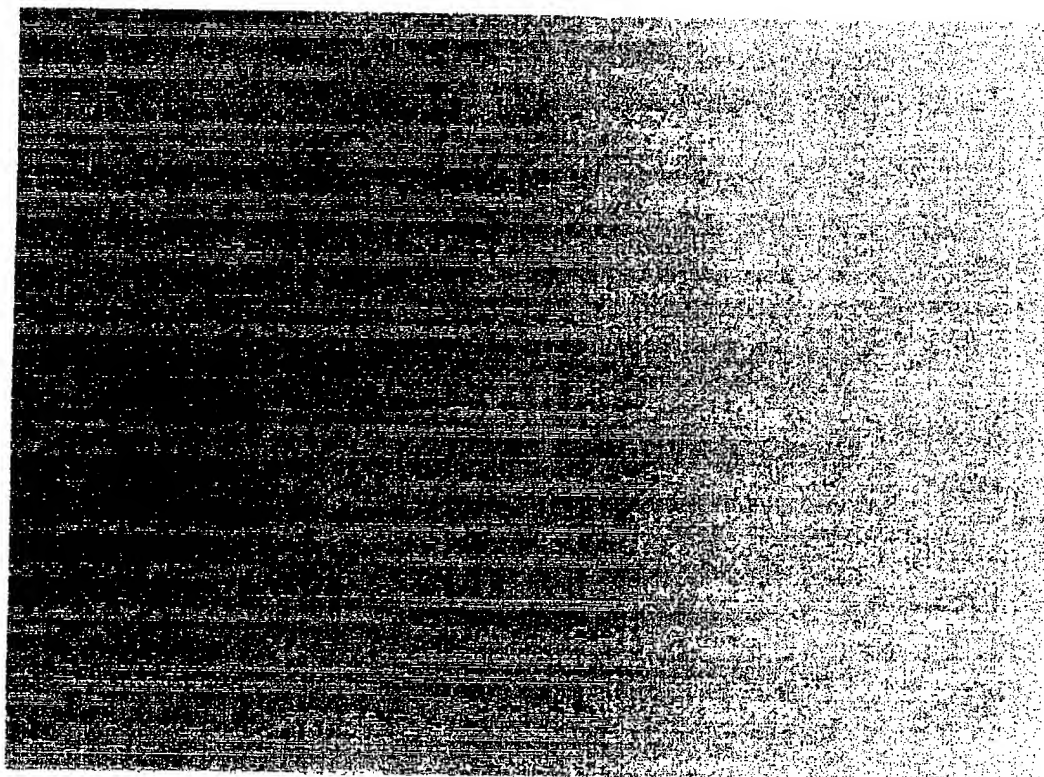


FIG. 11B

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09941492.040402

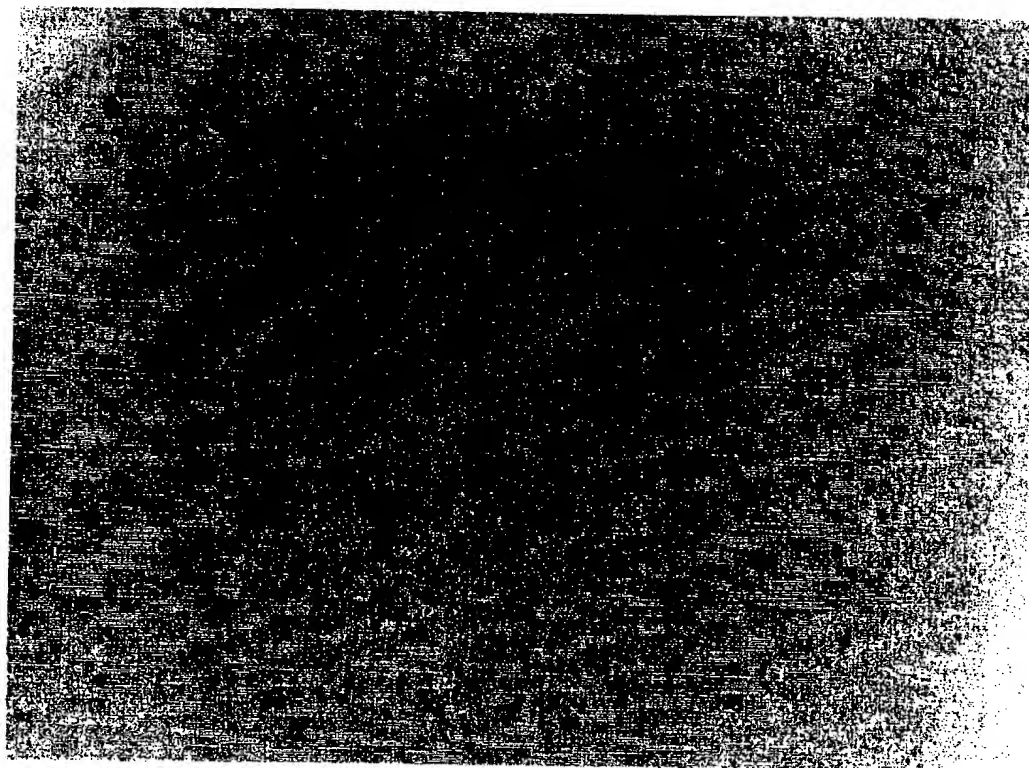


FIG.11C

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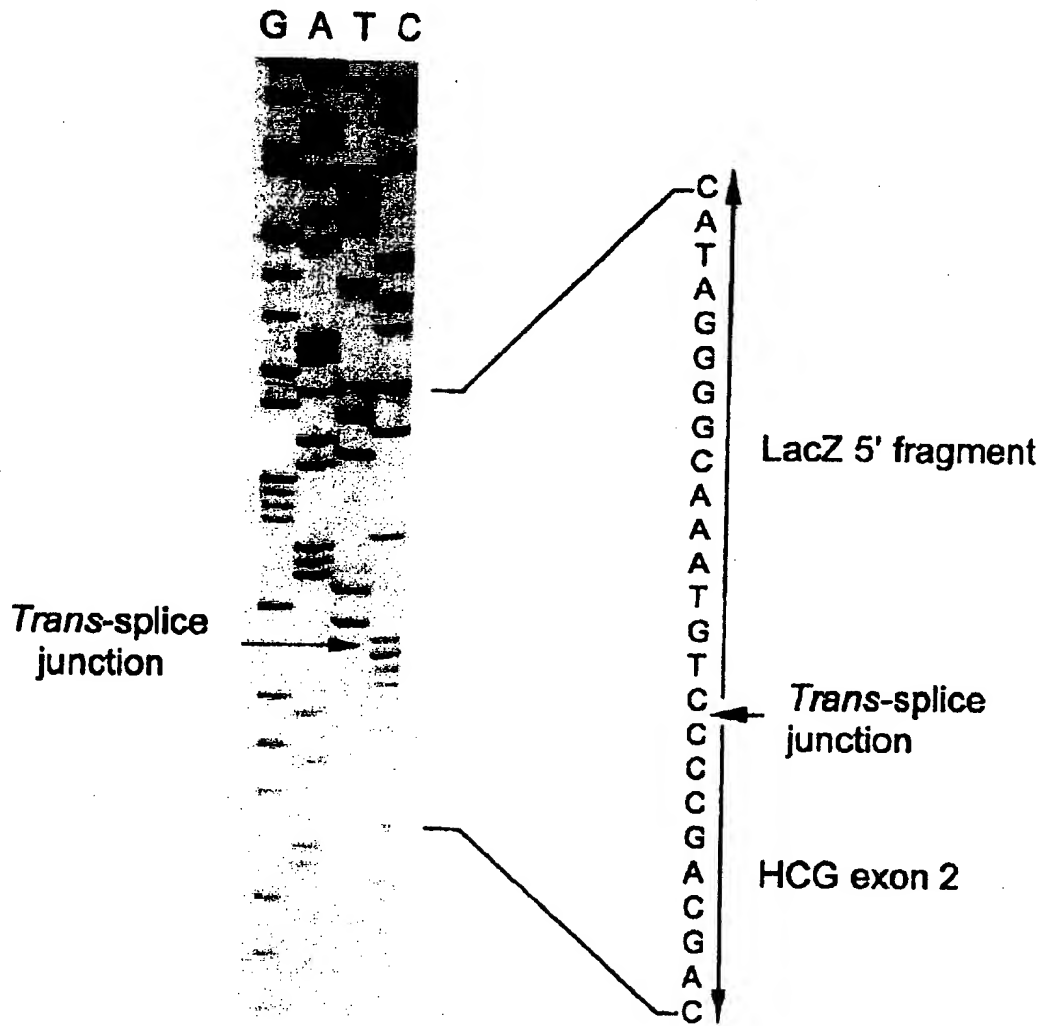
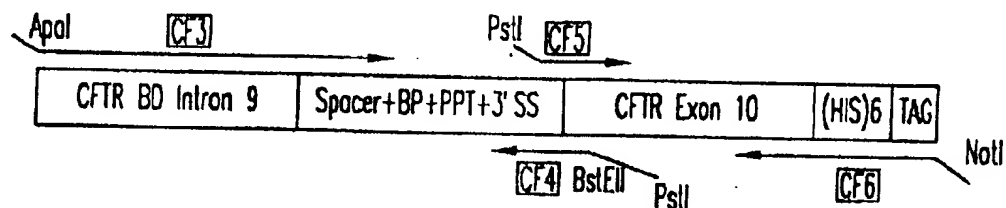


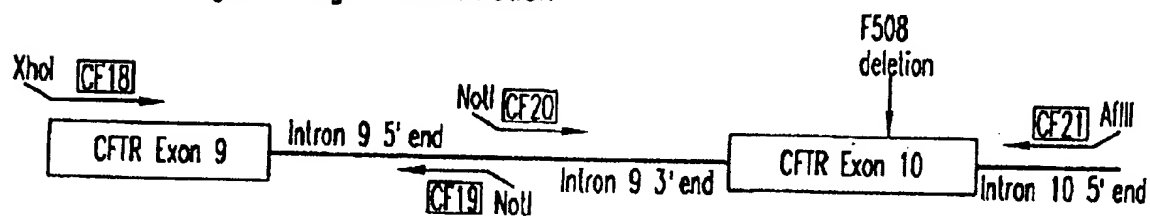
FIG.12A

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CFTR Pre-therapeutic molecule (PTM or "bullet")



CFTR mini-gene target-construction



Trans-splicing Repair

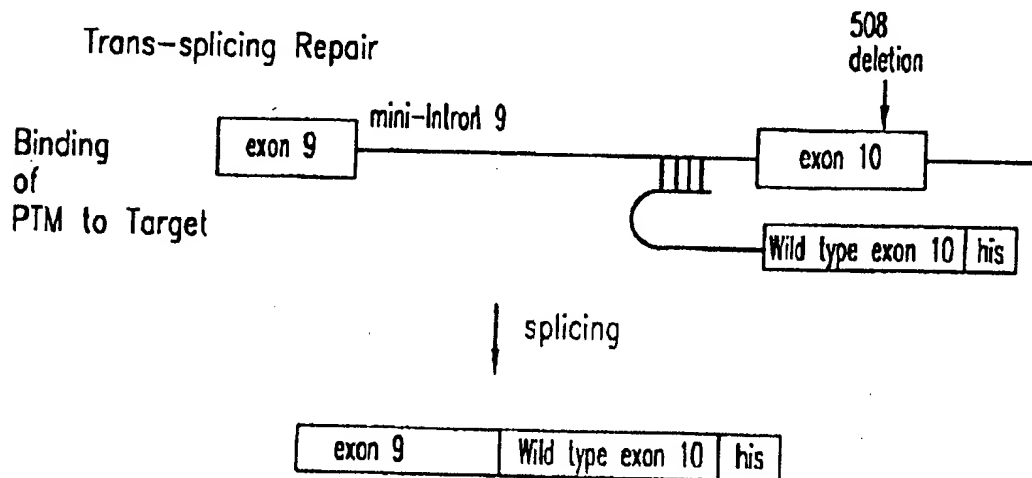


FIG.13

204070" 25474660

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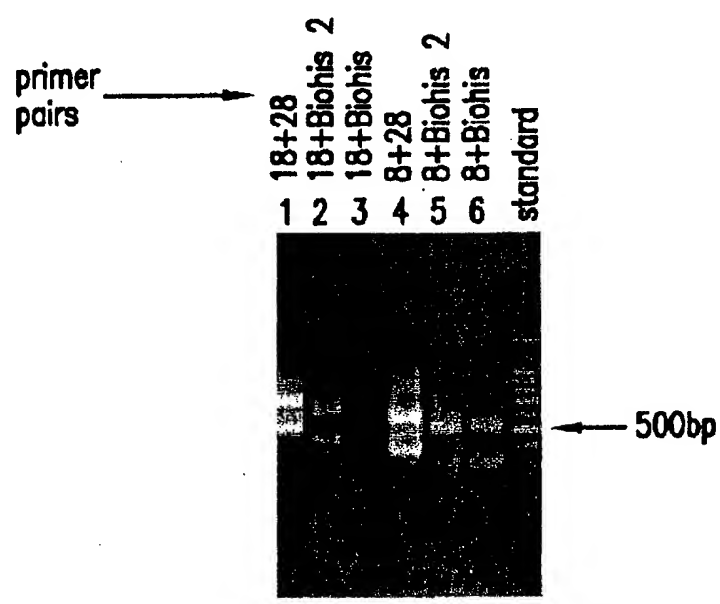
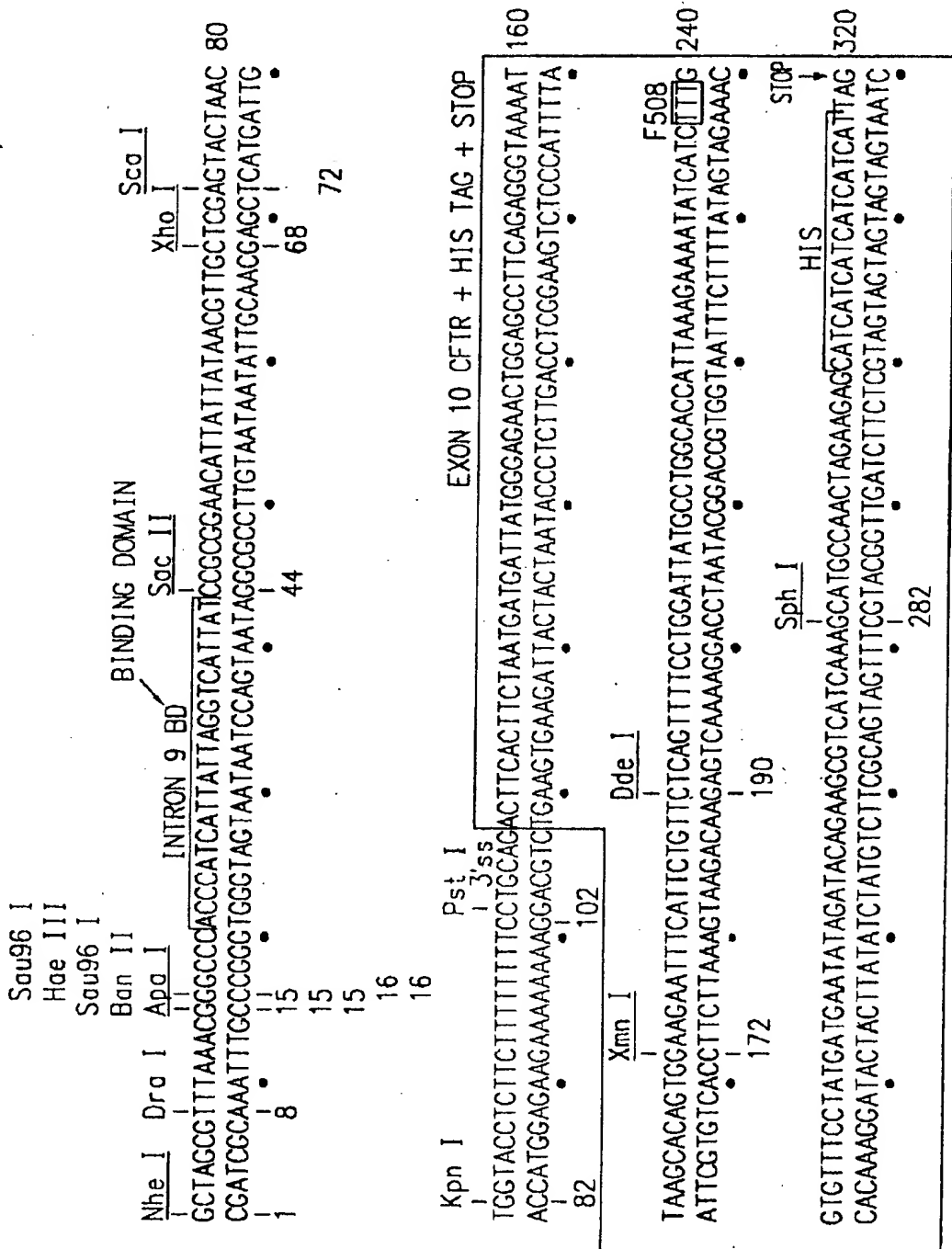


FIG.14

24 8 91

DNA sequence 500 b.p. GCTAGCGTTTAA ... TGGCACTCCAC linear

Positions of Restriction Endonucleases sites (unique sites underlined)



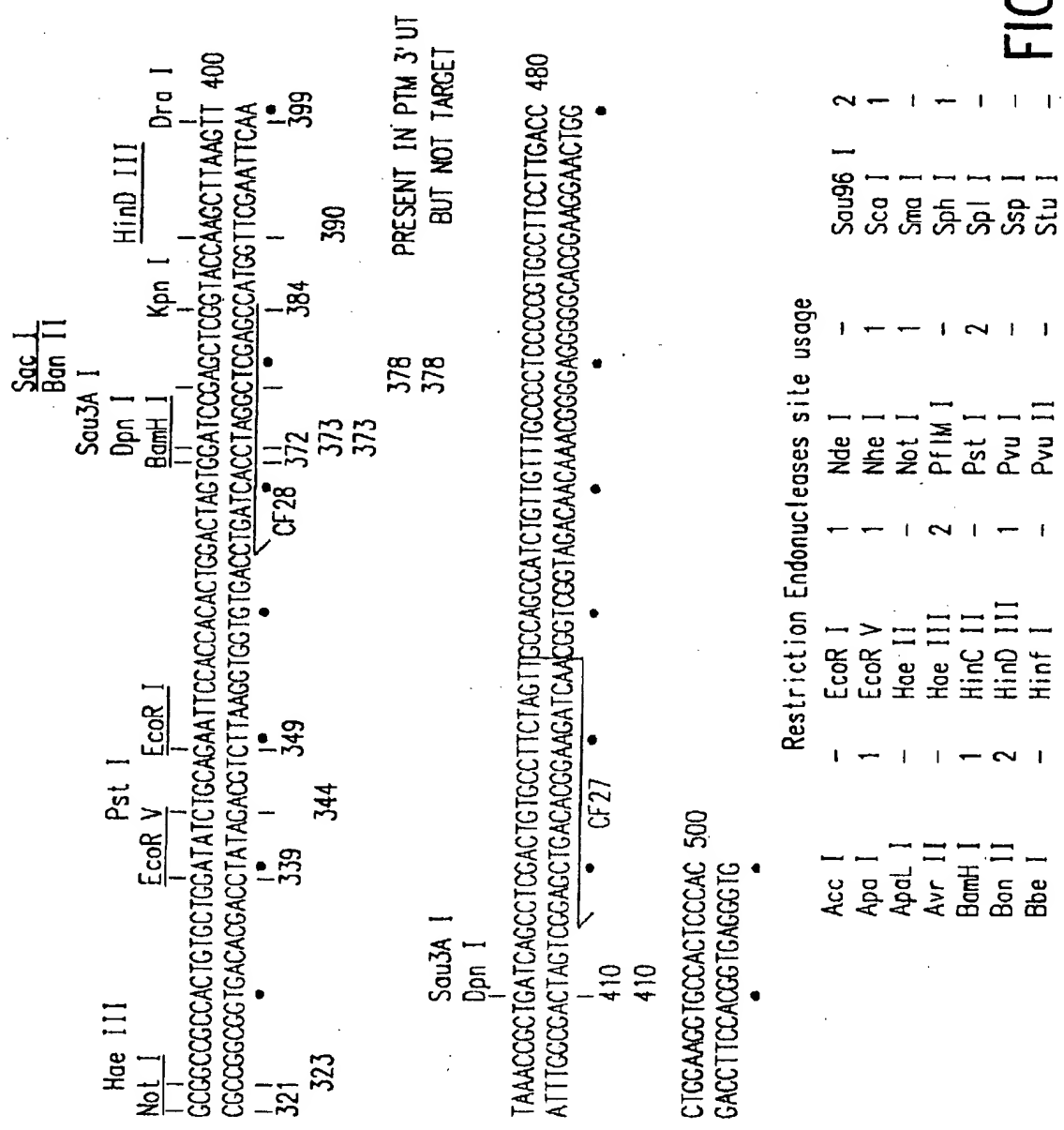


FIG.15B

Restriction Endonucleases site usage

Acc I	-	EcoR I	1	Nde I	-	Sau96 I	2
Apa I	1	EcoR V	1	Nhe I	1	Sca I	1
ApaL I	-	Hae II	-	Not I	1	Sma I	-
Avr II	-	Hae III	2	PfIM I	-	Sph I	1
BamH I	1	HinC II	-	Pst I	2	Spl I	-
Bon II	2	HinD III	1	Pvu I	-	Ssp I	-
Bbe I	-	Hinf I	-	Pvu II	-	Stu I	-

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+

CFTR Target
(mini-gene)



F508 deletion

Mini-intron 9
(~0.6kb)

Cotransfect PTM and target molecules in HEK 293 cells
and detect repaired CFTR mRNA by RT-PCR.

Repaired
CFTR mRNA

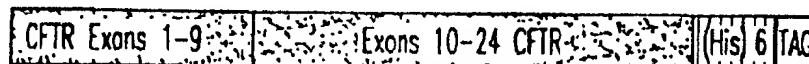


FIG.16

204040" 26474660

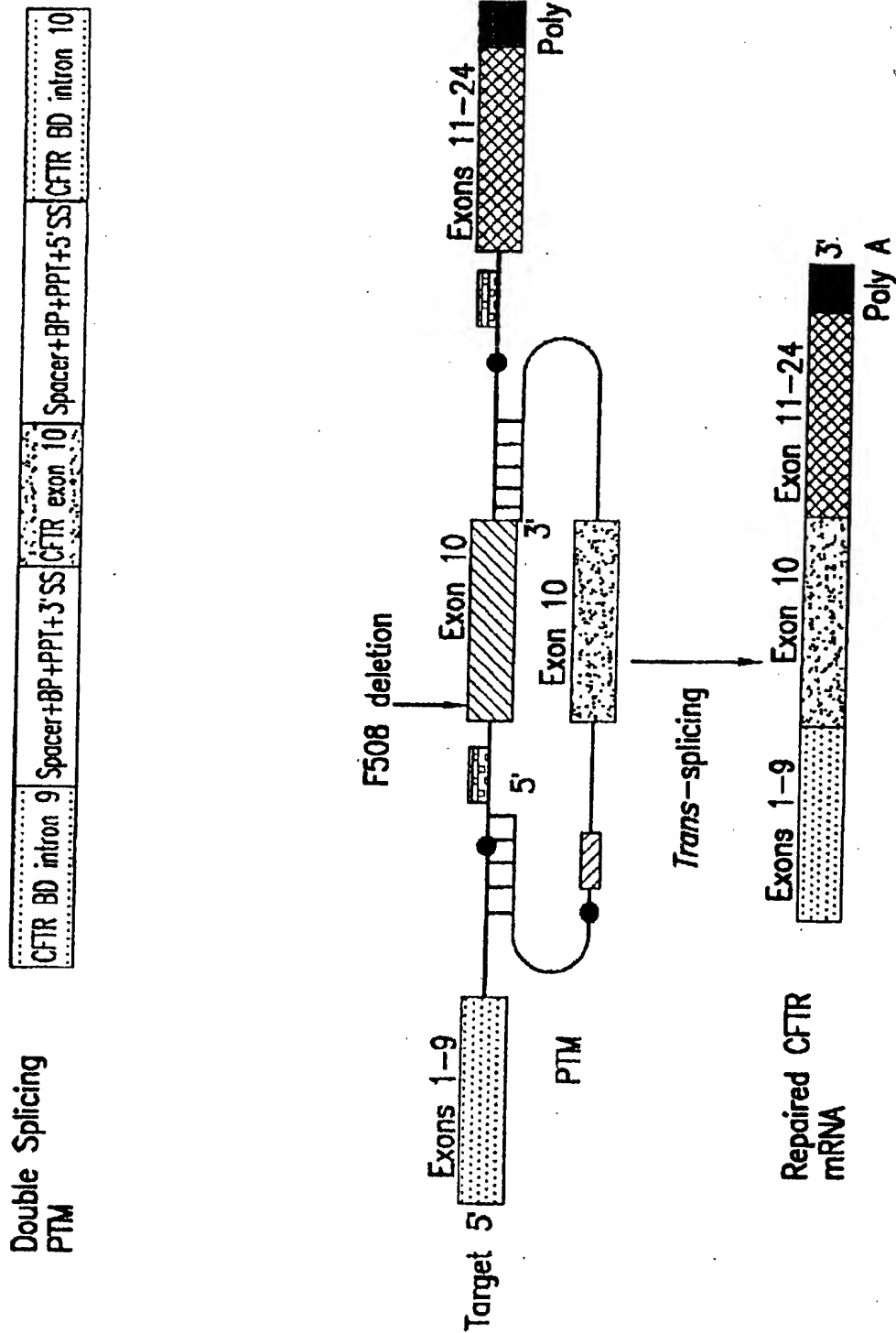


FIG.17

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DOUBLE TRANS-SPLICING SPECIFIC TARGET

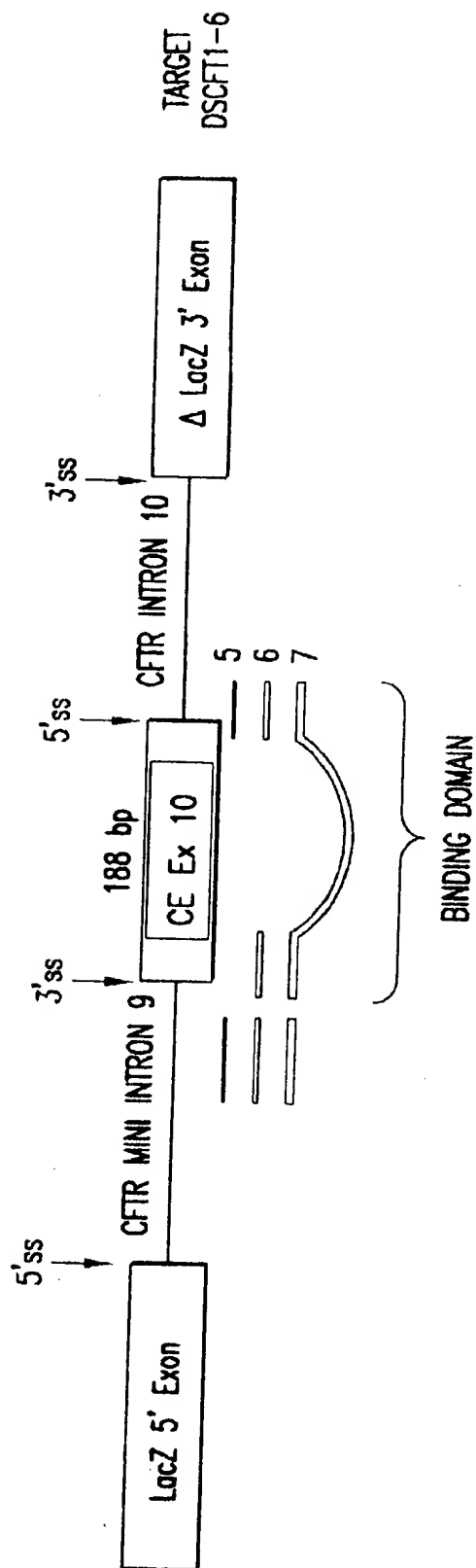


FIG.18

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DOUBLE TRANS-SPLICING PTMS

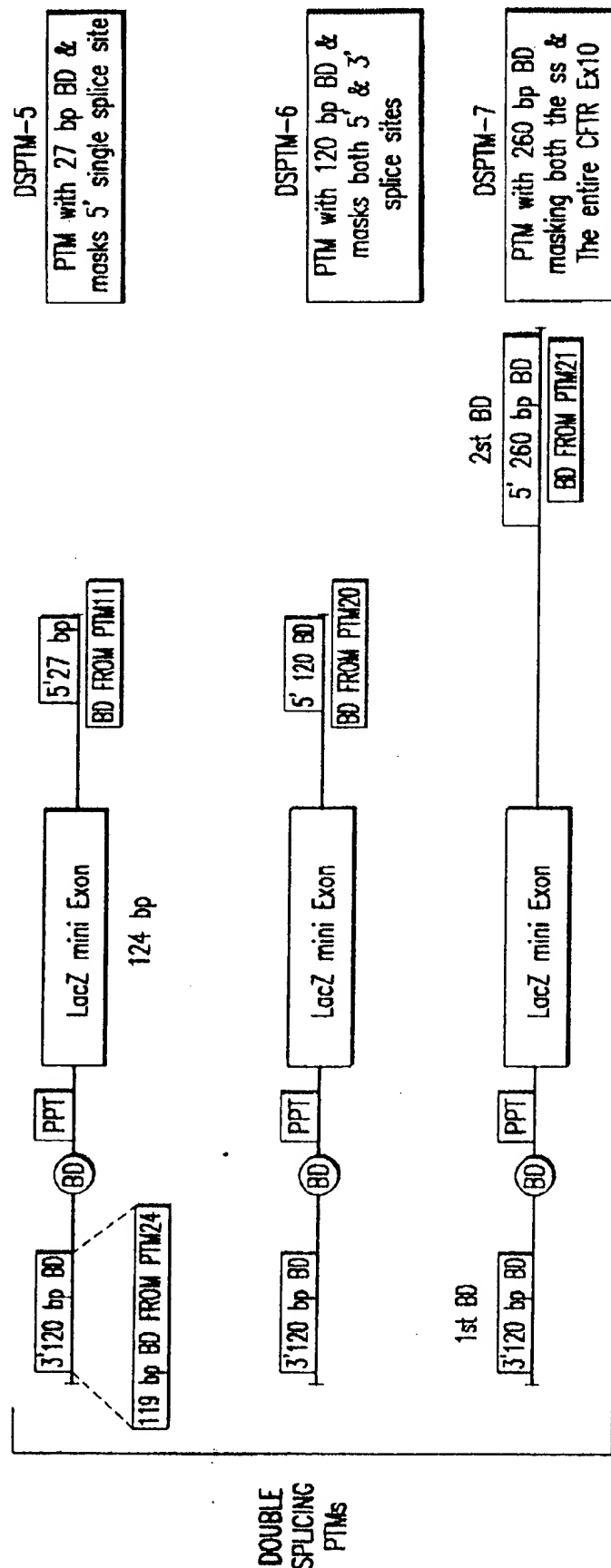
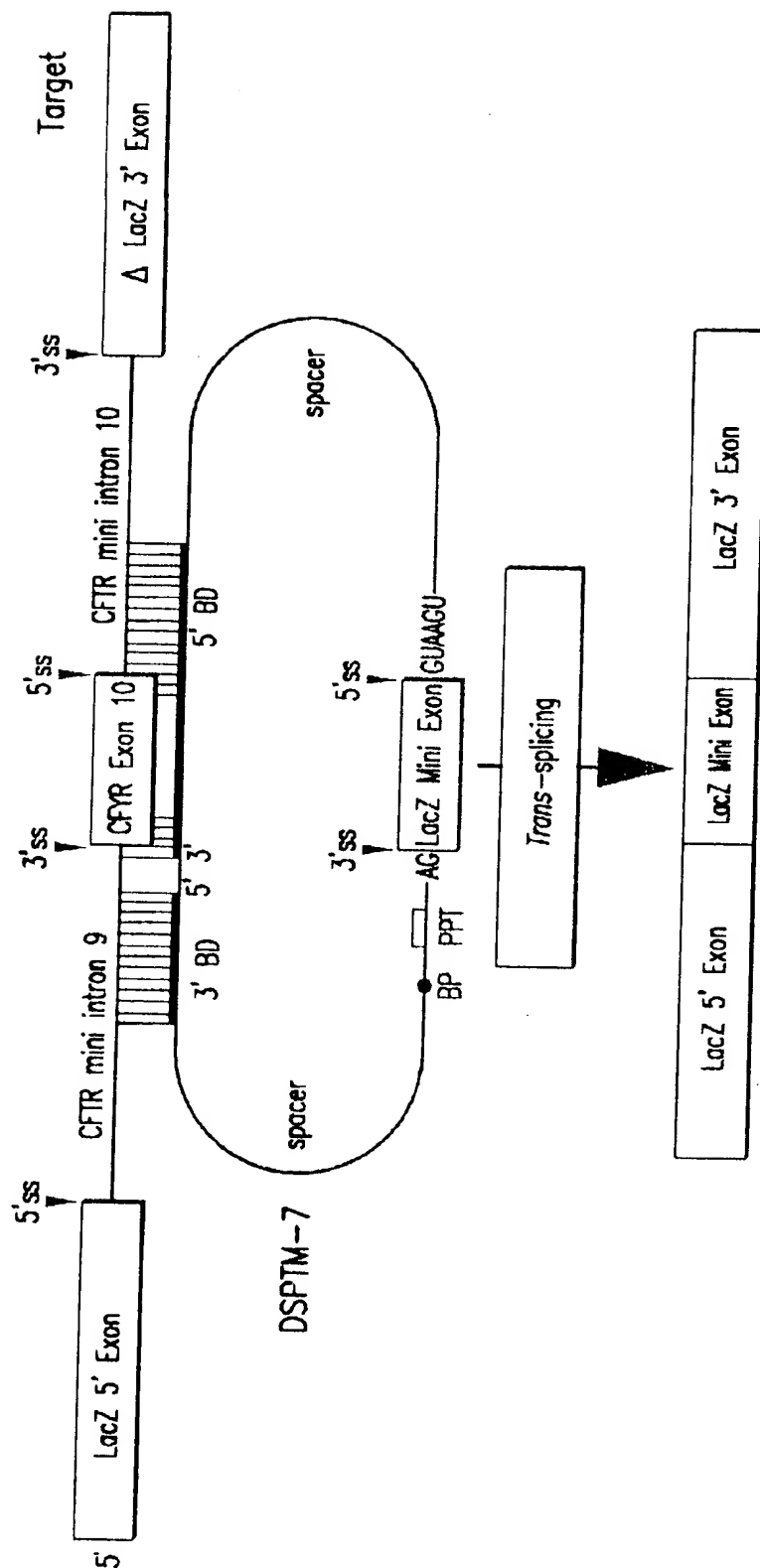


FIG.19

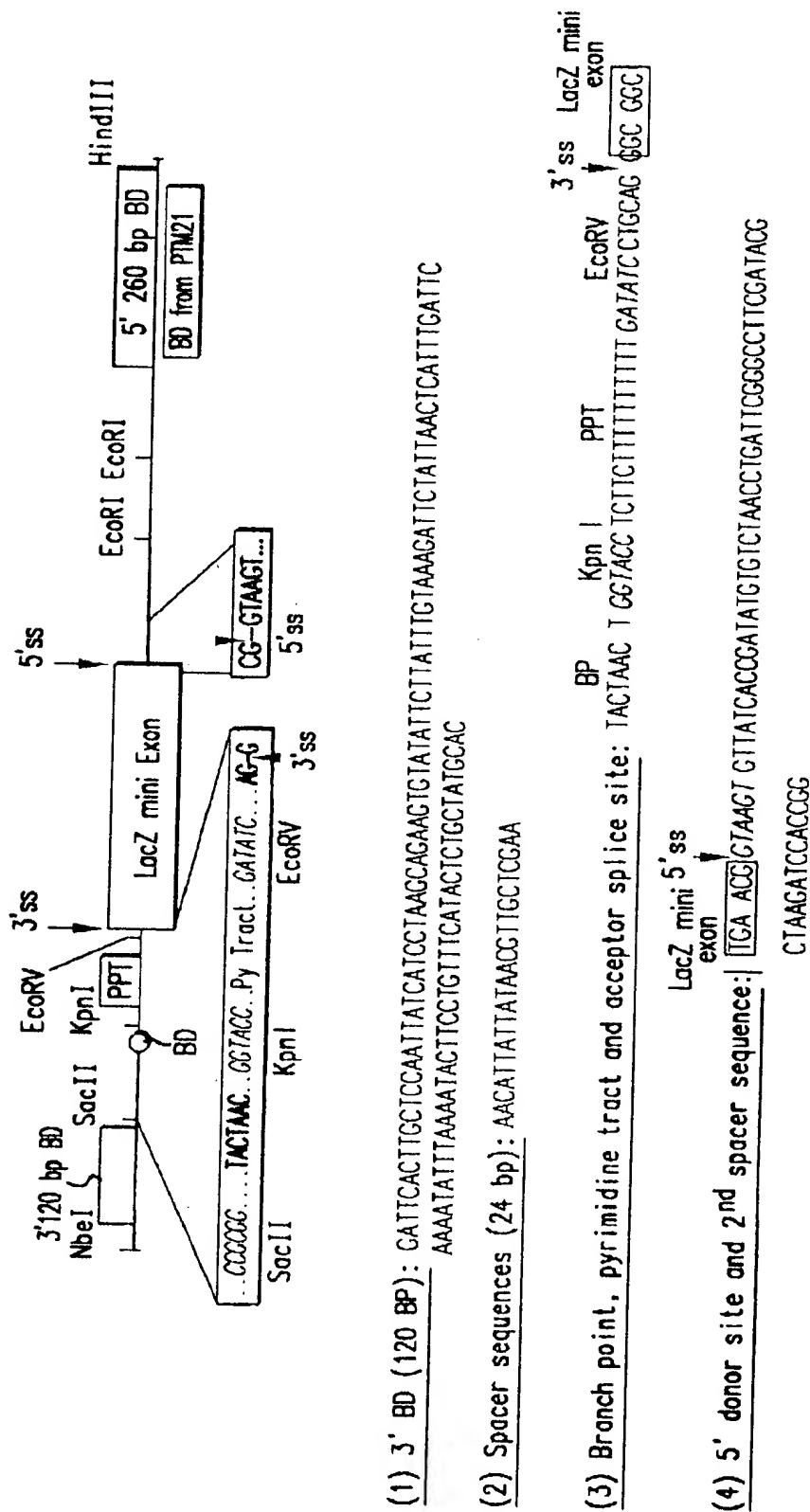
204040" 2577450

DOUBLE TRANS-SPLICING β -GAL MODEL



Repaired LacZ mRNA

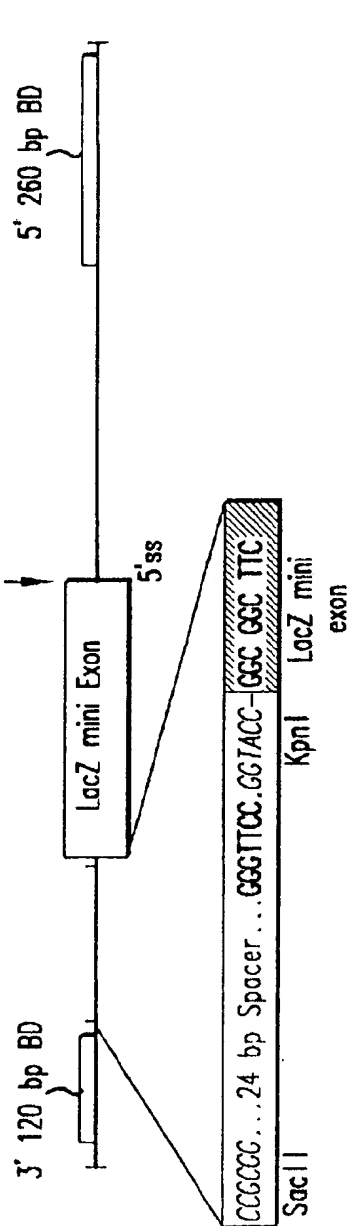
FIG.20



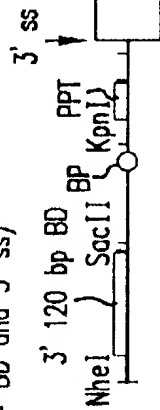
(5) 5' BD (260 BP): TCAAAAGCTTTCACATAAATTTTACTCTCTTGAAATTCATGCTTTCATCAGCCTTCGTATCIAIATTCATCATTGGAACACCAATGATTTTTCTTTAATGGTCCCTGGCATAATCTGSAAACTGATAACACAATGAAATCTTCCACTGTGCTTAA
AAAAACCTTCGAAATCTCCATTAATCATCATTTACAACGTGAACCTCGGAAATAAAACCCATCATTTAATCACTCATCAAAATCACGC

FIG. 21

DSPTM8: (Δ 3' ss: 3' splice elements i.e. BP, PPT & AG dinucleotide has been deleted and replaced with random sequences, but still has the functional 5' splice site)



PTM29 (lacks 2nd BD and 5' ss)



PTM30 (lacks 1st BD and 3' ss)



FIG.22

Mutants

ACCURACY OF DOUBLE TRANS-SPICING REACTION

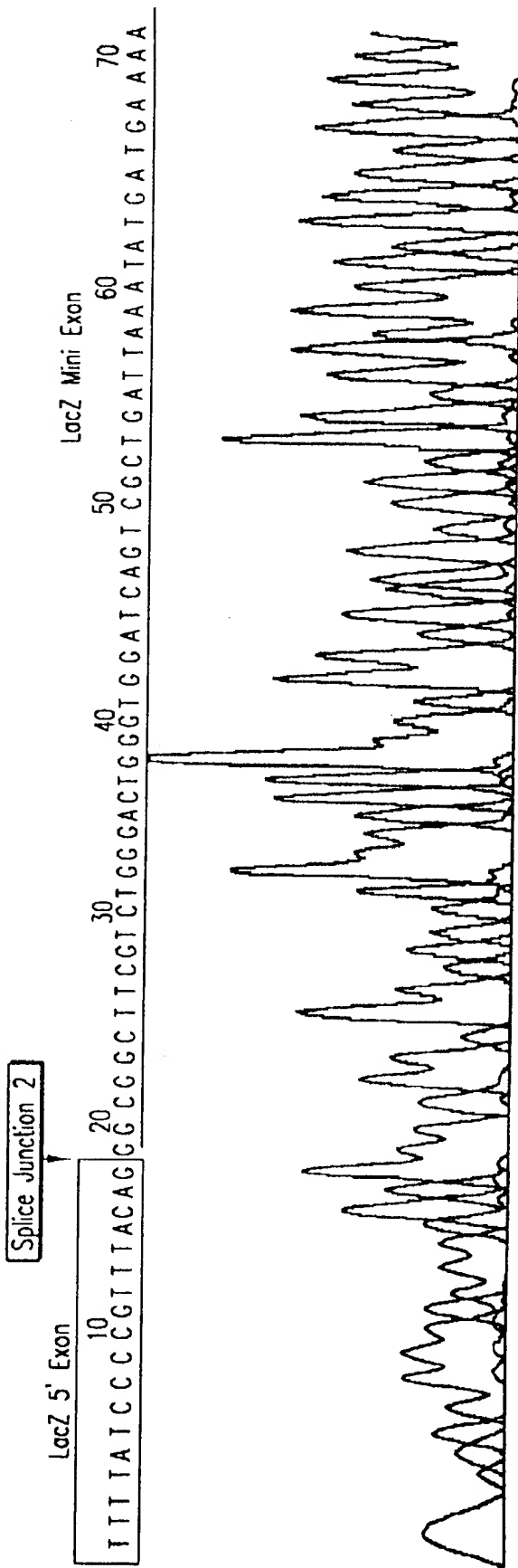


FIG.23A

ACCURACY OF DOUBLE TRANS-SPlicing REACTION

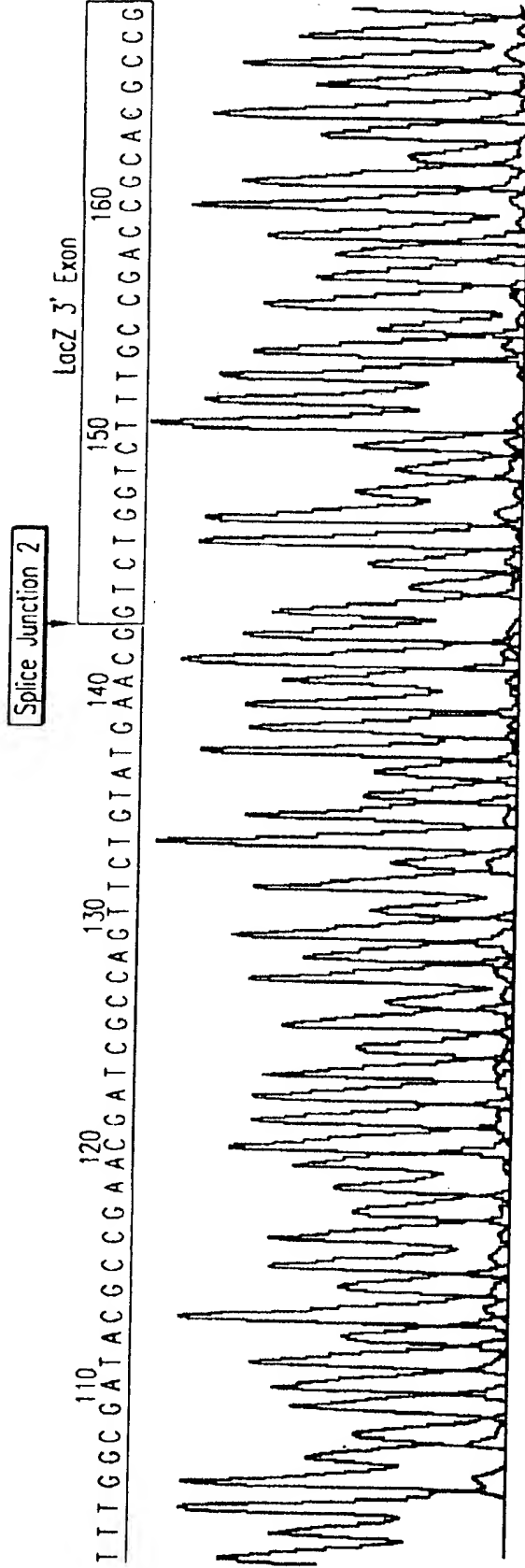
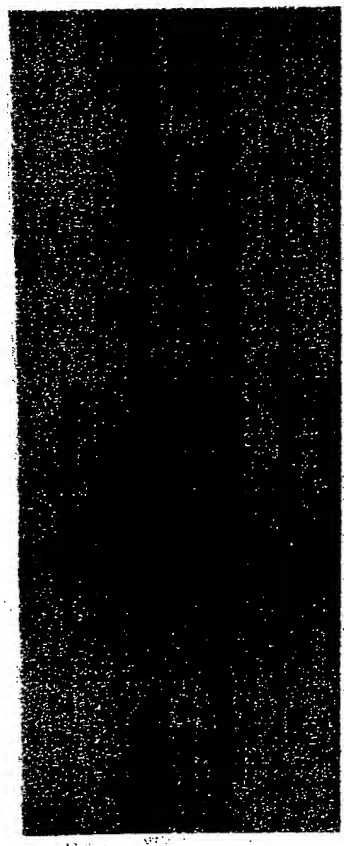


FIG.23B

Double Trans-splicing Produces Full-length Protein



β-gal →
(120 kDa)

1 2 3 4 5 6 7

Lane 1: DSCFT1.6 Target alone 25 μg
Lane 2: DSPTM7 25 μg
Lane 3 Target + PTM #6 25 μg
Lane 4: Target + PTM #9 25 μg
Lane 5: Delta 3' splice mutant alone 25 μg
Lane 6: Target + Delta 3' ss 25 μg
Lane 7: Target+PTM29+30 (mutants) 25 μg

Figure 24

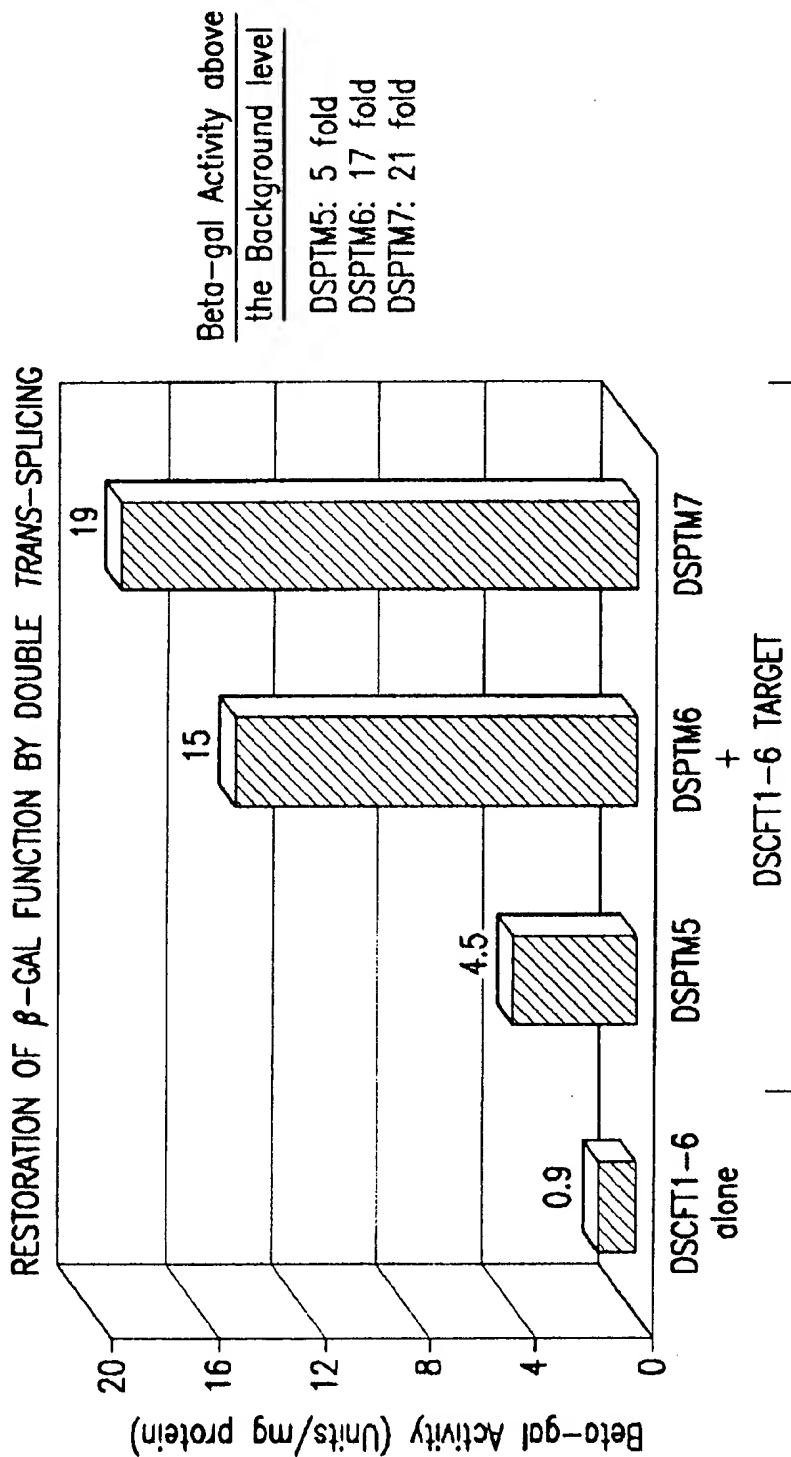


FIG.25

RESTORATION OF β -GAL ACTIVITY IS DUE TO DOUBLE RNA TRANS-SPLICING EVENTS

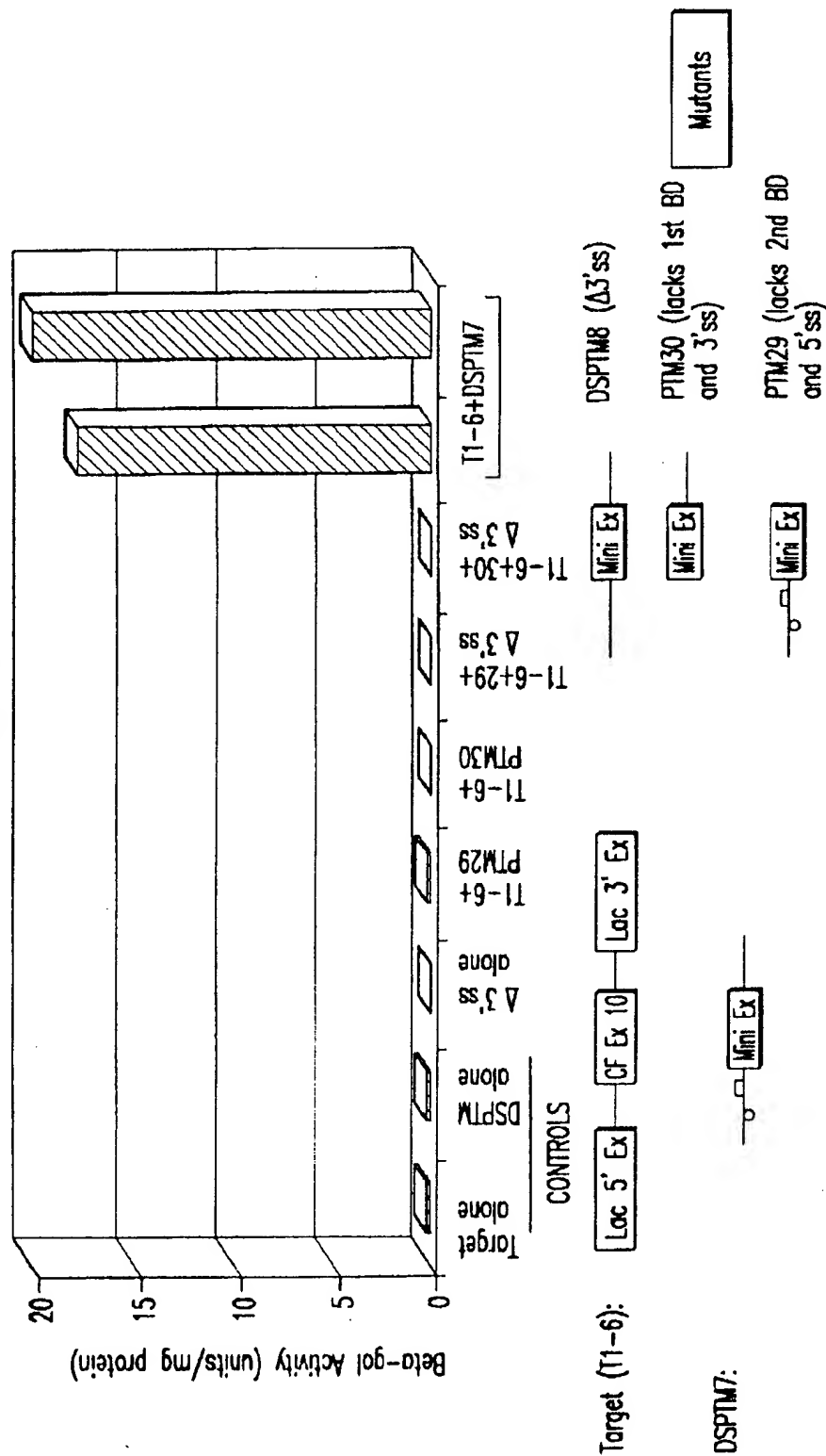


FIG.26

DOUBLE TRANS-SPLICING: TITRATION OF TARGET & PTM

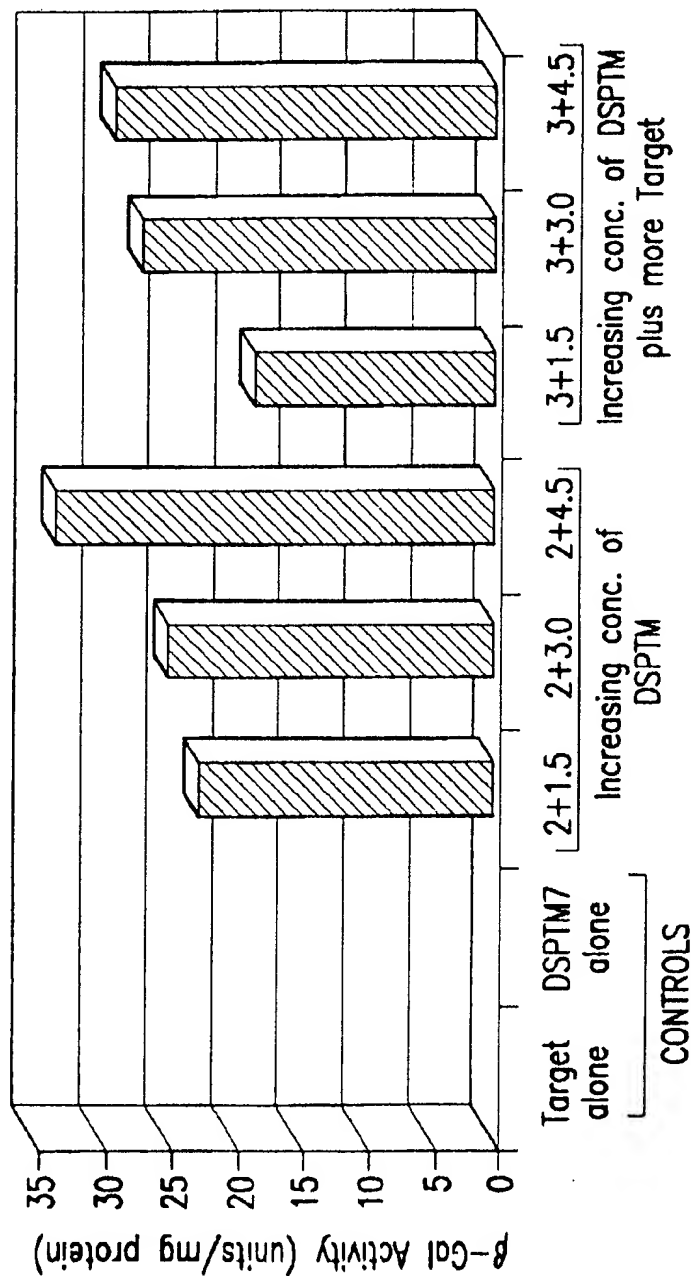


FIG.27

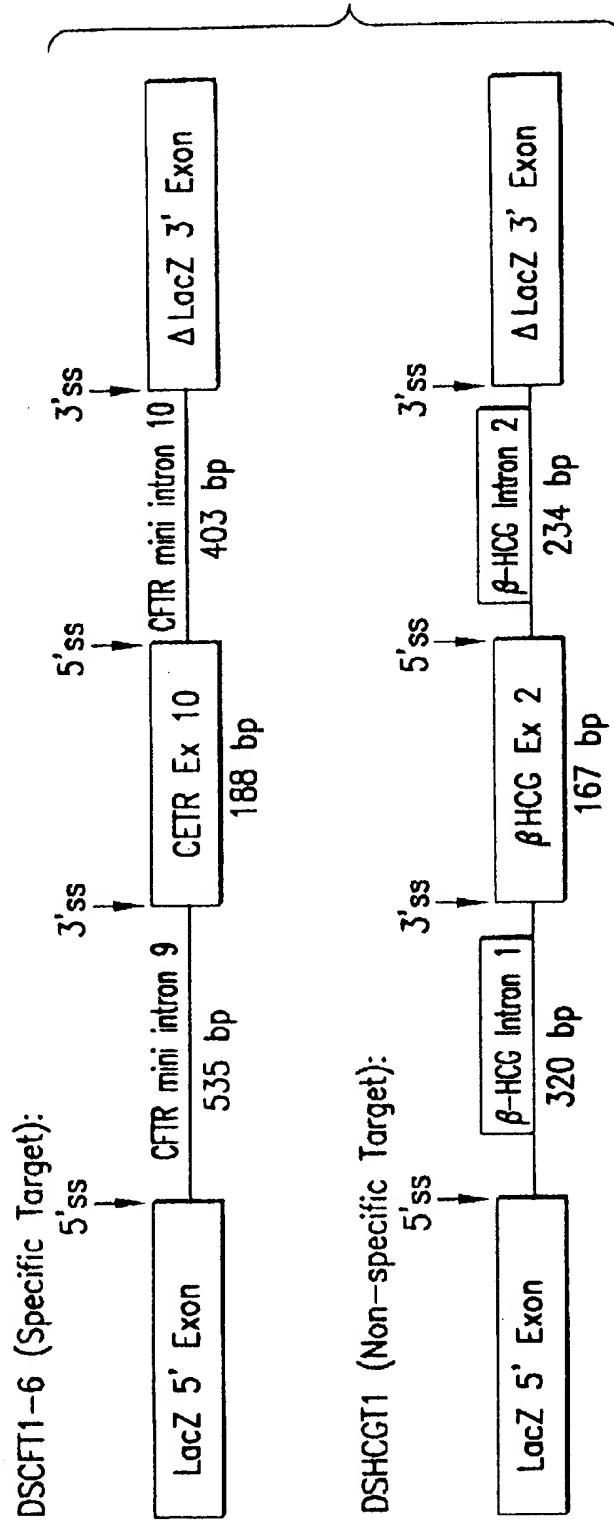


FIG.28

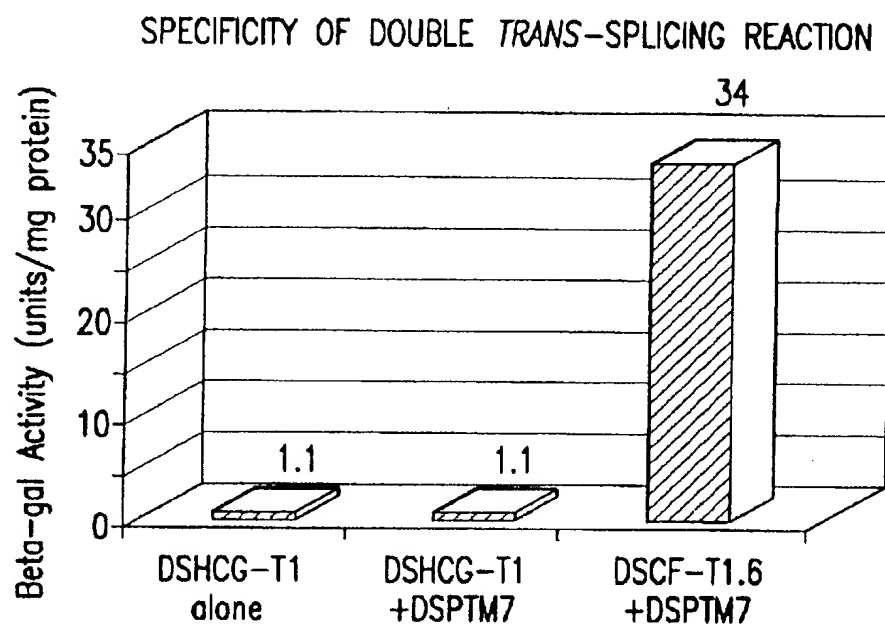


FIG.29

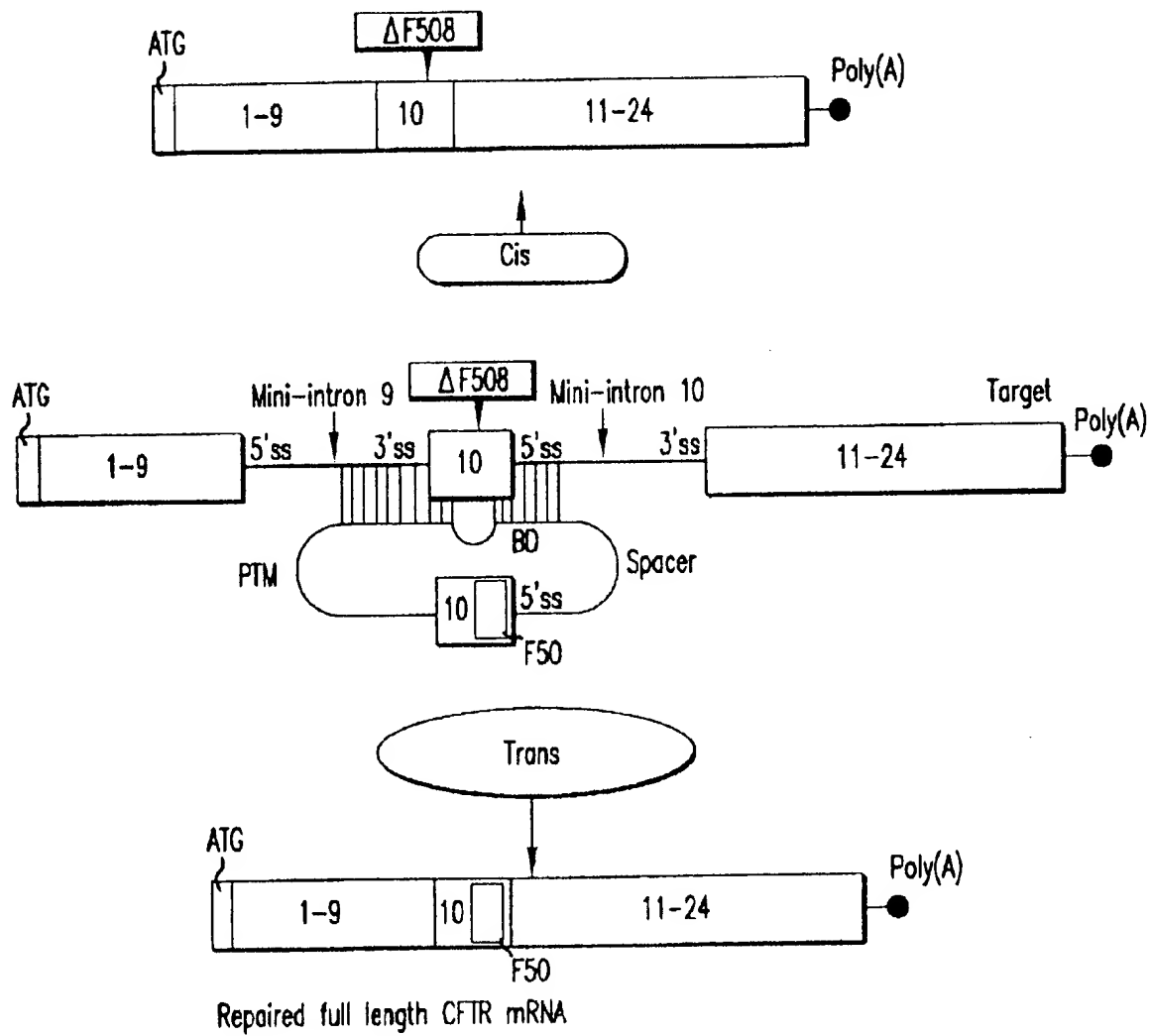
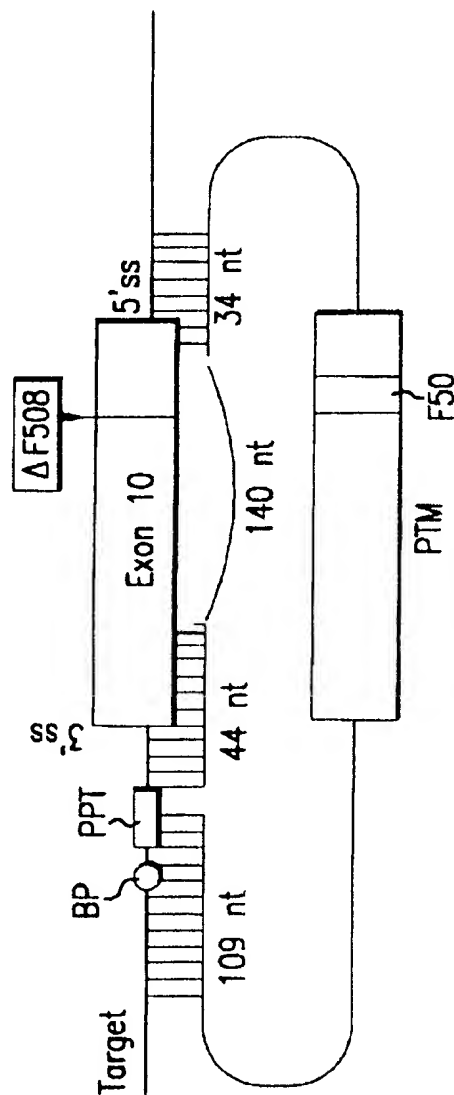


FIG.30

PTM with a long binding domain masking
two splice sites and part of exon 10
in a mini-gene target



ACGAGCTTGGCTCATGATGATGGCGGAGTTAGAACCAAGTGAAGGCAAGATCAACATTCGG
GCCGATCAGCTTTTCAGCCAAATTCAGTGGATCATGCCCGGTACCATCAAGGAGAACATTAAT
CTTCGCGCTCAGTACGACGAGTACCGCTATCGCTGGTGAATTAAGCCCTGTCAGTTCGAGGAG

MCU in exon 10 of PTM

88 OF 192 (46%) bases in PTM exon 10 are not complementary to
its binding domain (bold and underlined).

FIG.31

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Sequence of a double
Trans-spliced product

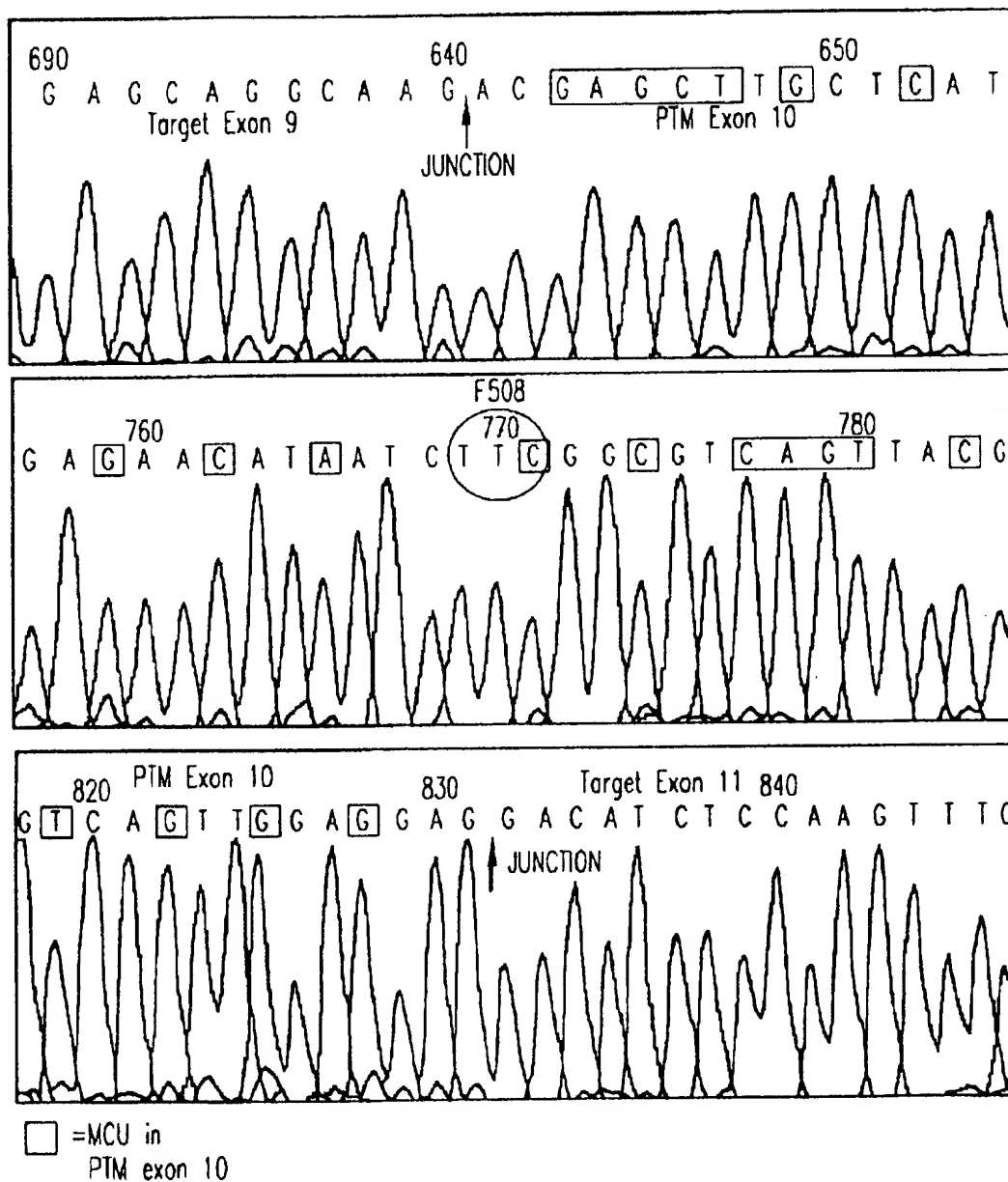


FIG.32

204040" 2544660

CF-TR Repair: 5' Exon-Replacement schematic diagram of a PTM binding to the splice site of intron 10 of a mini-gene target

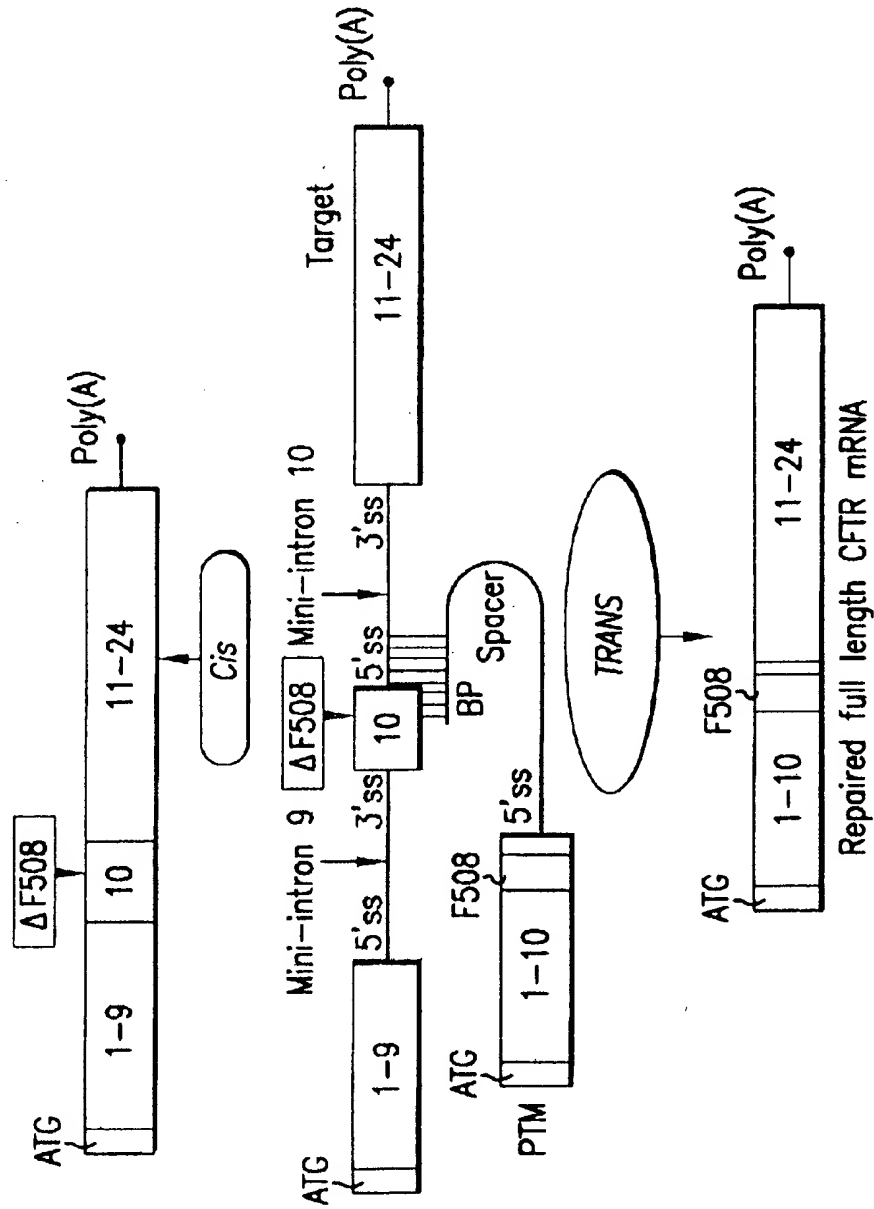


FIG.33

PTM with a short binding domain masking a single splice site in a mini-gene target.

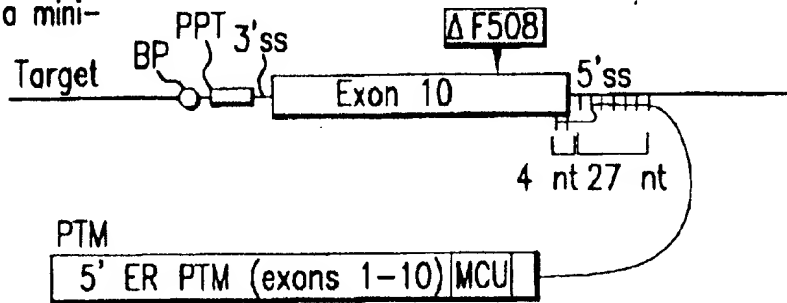


FIG.34A

PTM with a long binding domain masking two splice sites in a mini-gene target.

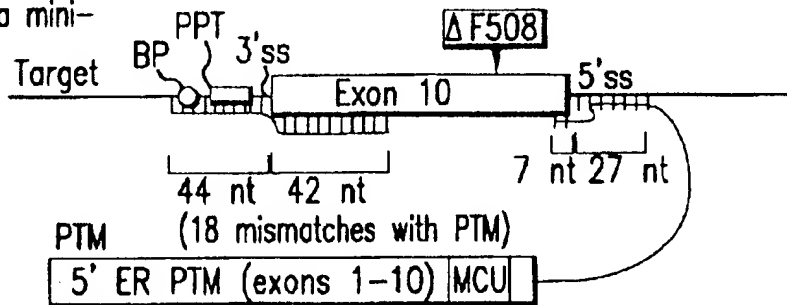


FIG.34B

PTM with a long binding domain masking two splice sites and the whole of exon 10 in a mini-gene target.

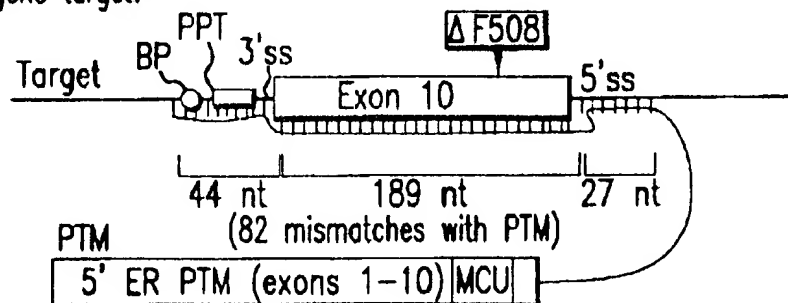
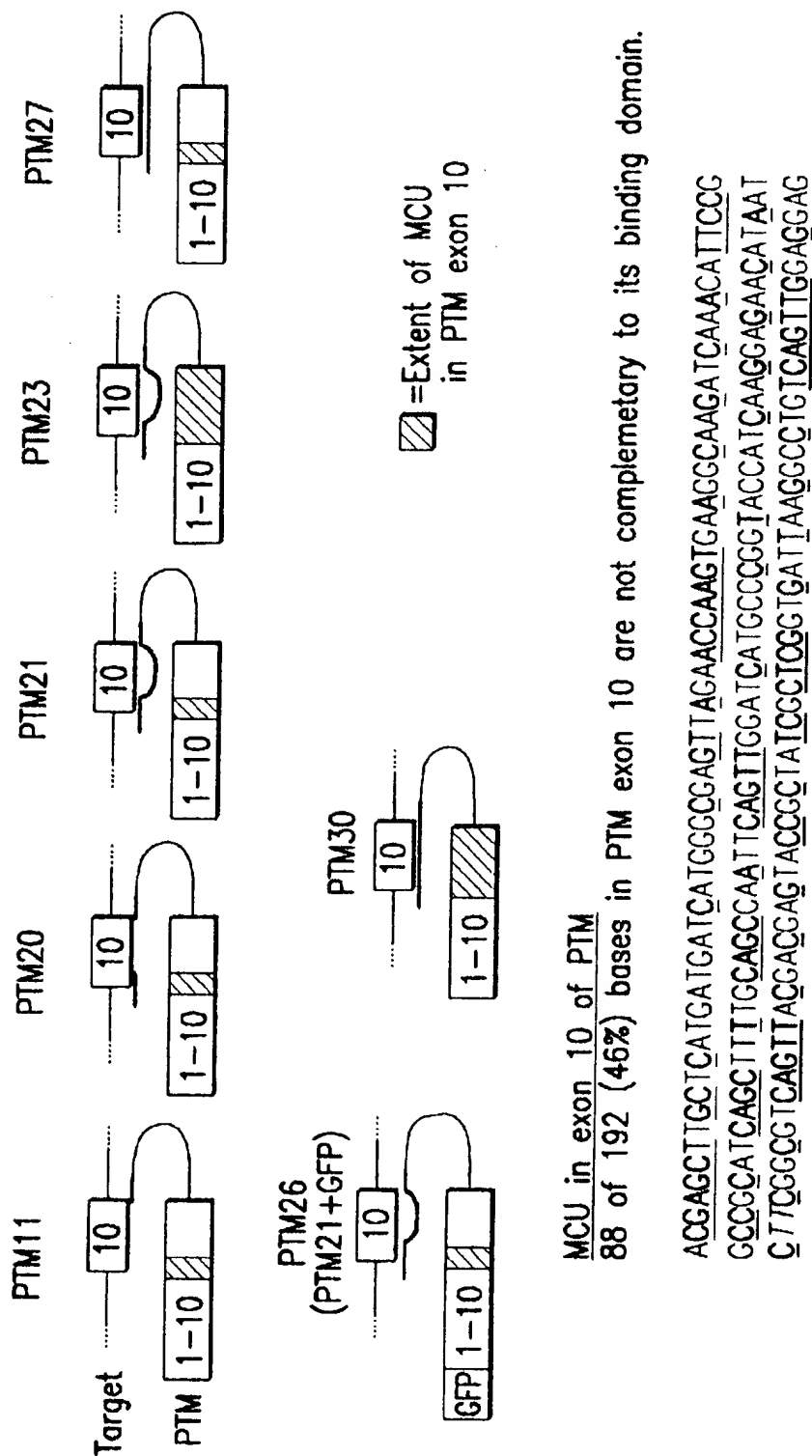


FIG.34C



MCU in exon 10 of PTM
88 of 192 (46%) bases in PTM exon 10 are not complementary to its binding domain.

ACGAGCTTGC¹TATGATCATGGCGAGTTACAACCAAGTGAAGCCAAGATCAAACA²TTCCG
GCGGCA³TCAGC⁴TTTCCAGCCAA⁵TTCAG⁶TTGGATCATGCCGGTACCATCAAGGAGAA⁷CATA⁸T
C⁹TTGGCG¹⁰TCAG¹¹TTACGACGAG¹²TACCG¹³CTATCCCTGGTGA¹⁴TAAAGCC¹⁵TCTCAG¹⁶TTGGAGGAG¹⁷

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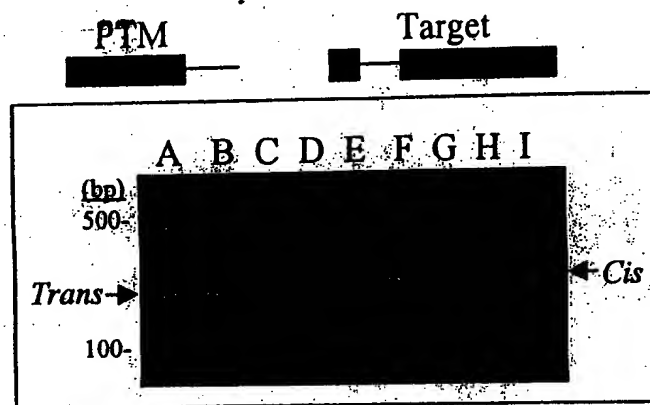


FIG. 36 A

204040" 26TH650

Cis-spliced product
[Primers CF1+CF111]

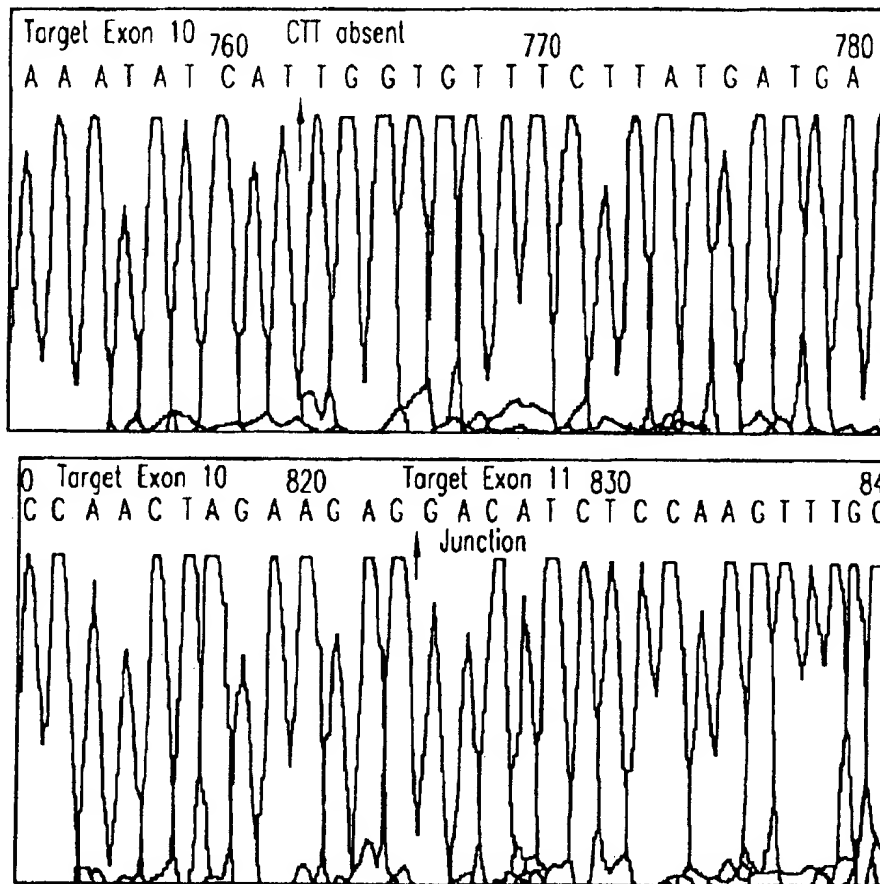


FIG.36A-1

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Trans-spliced product
[Primers CF93+CF111]

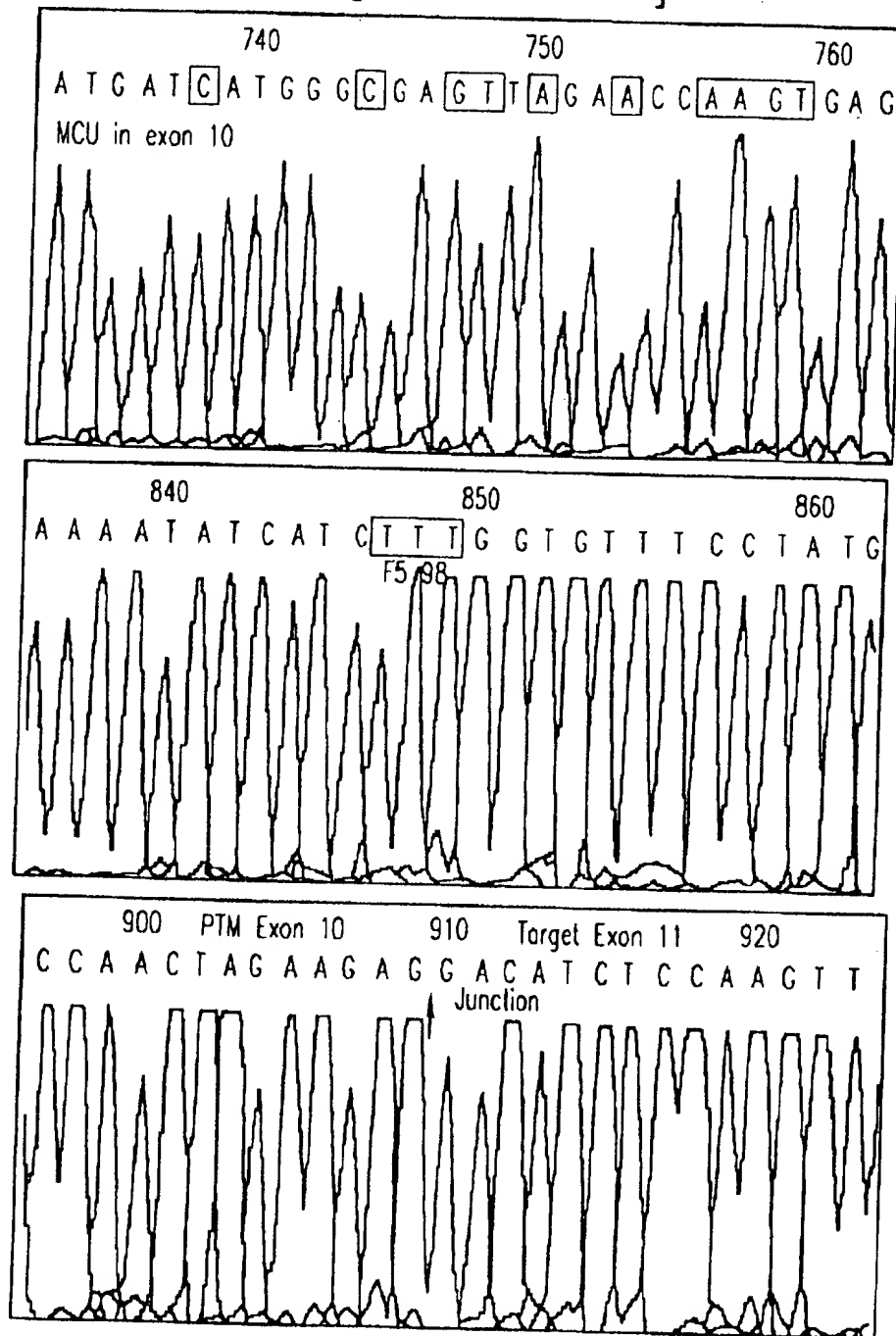


FIG.36B

201010 25449 0440

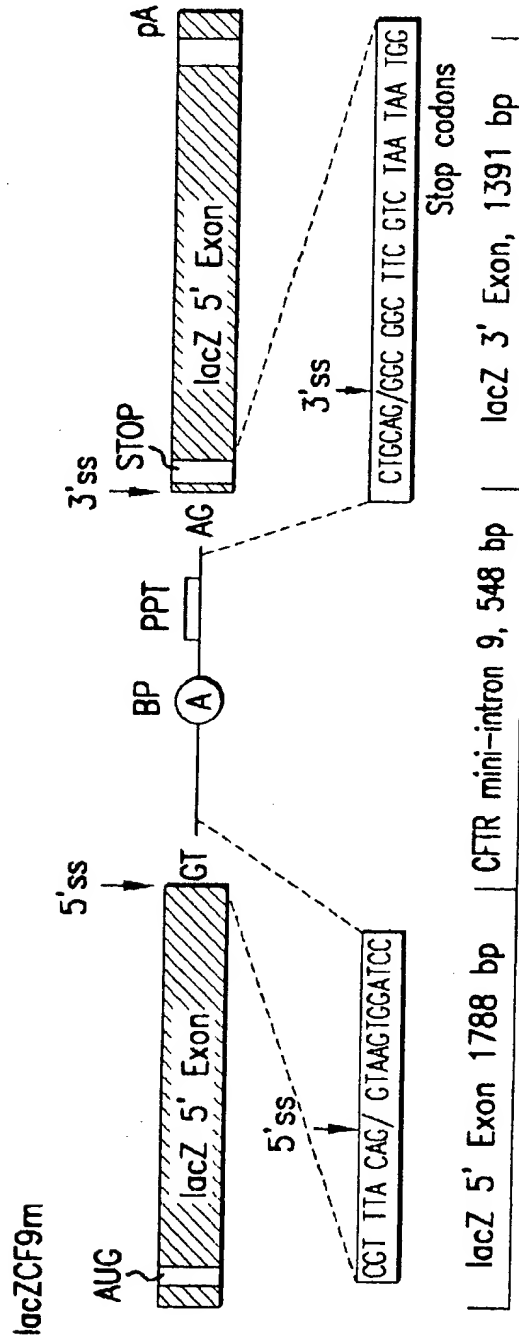


FIG.37A

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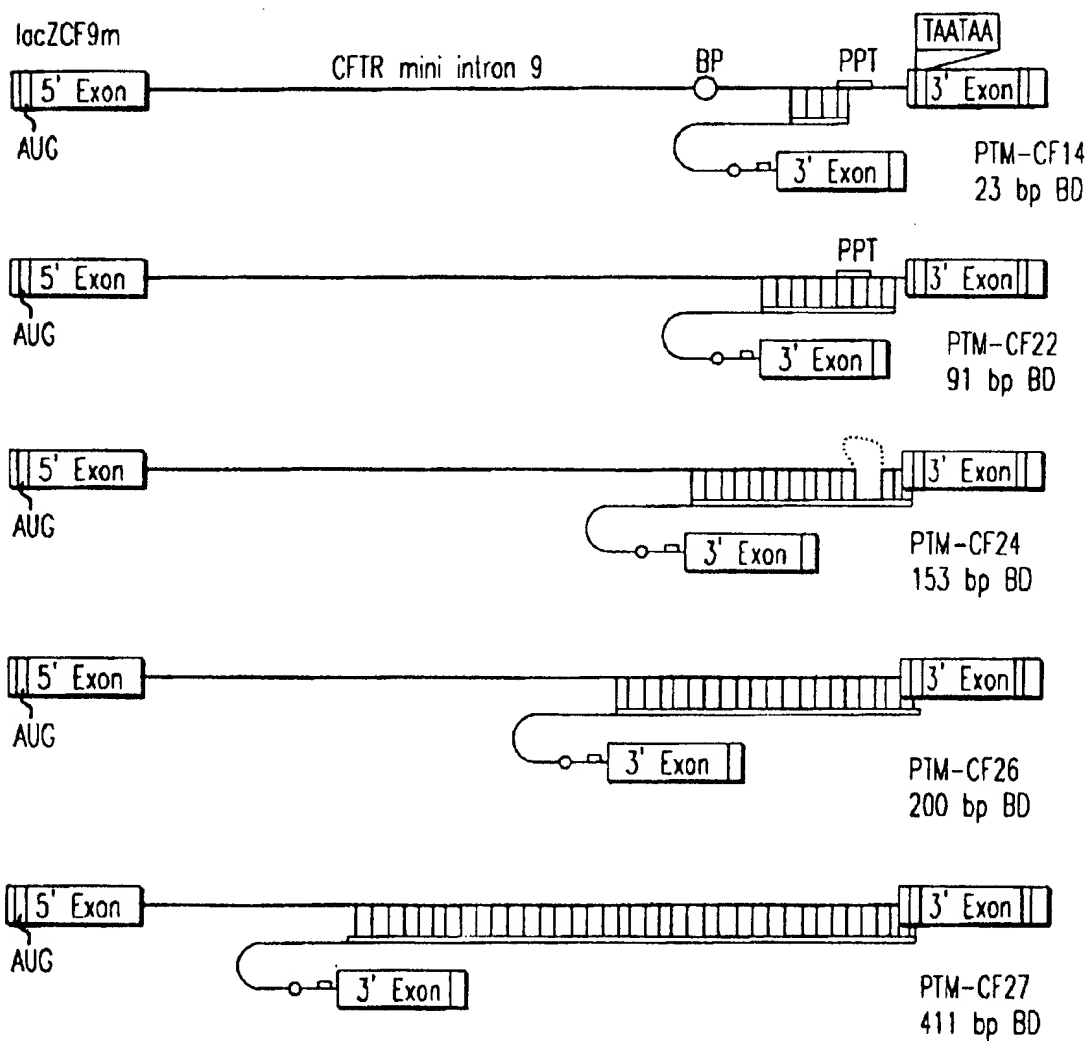
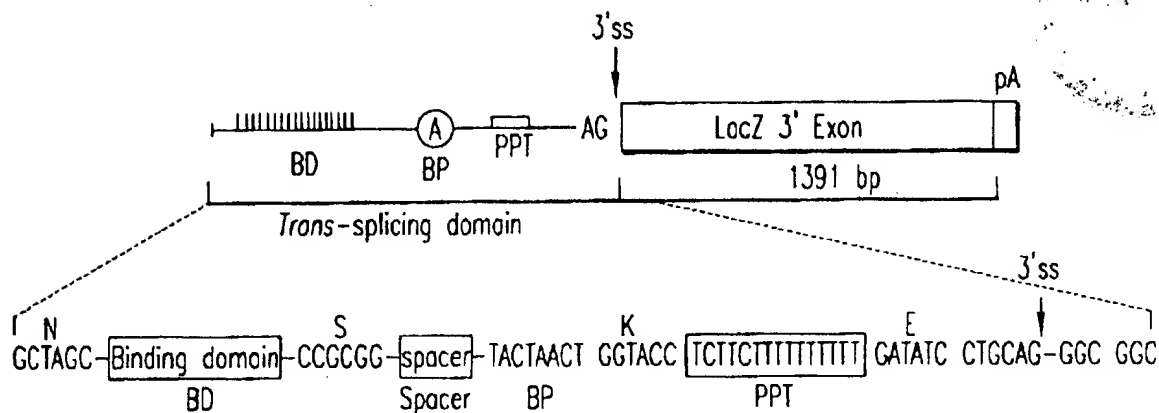


FIG.37B

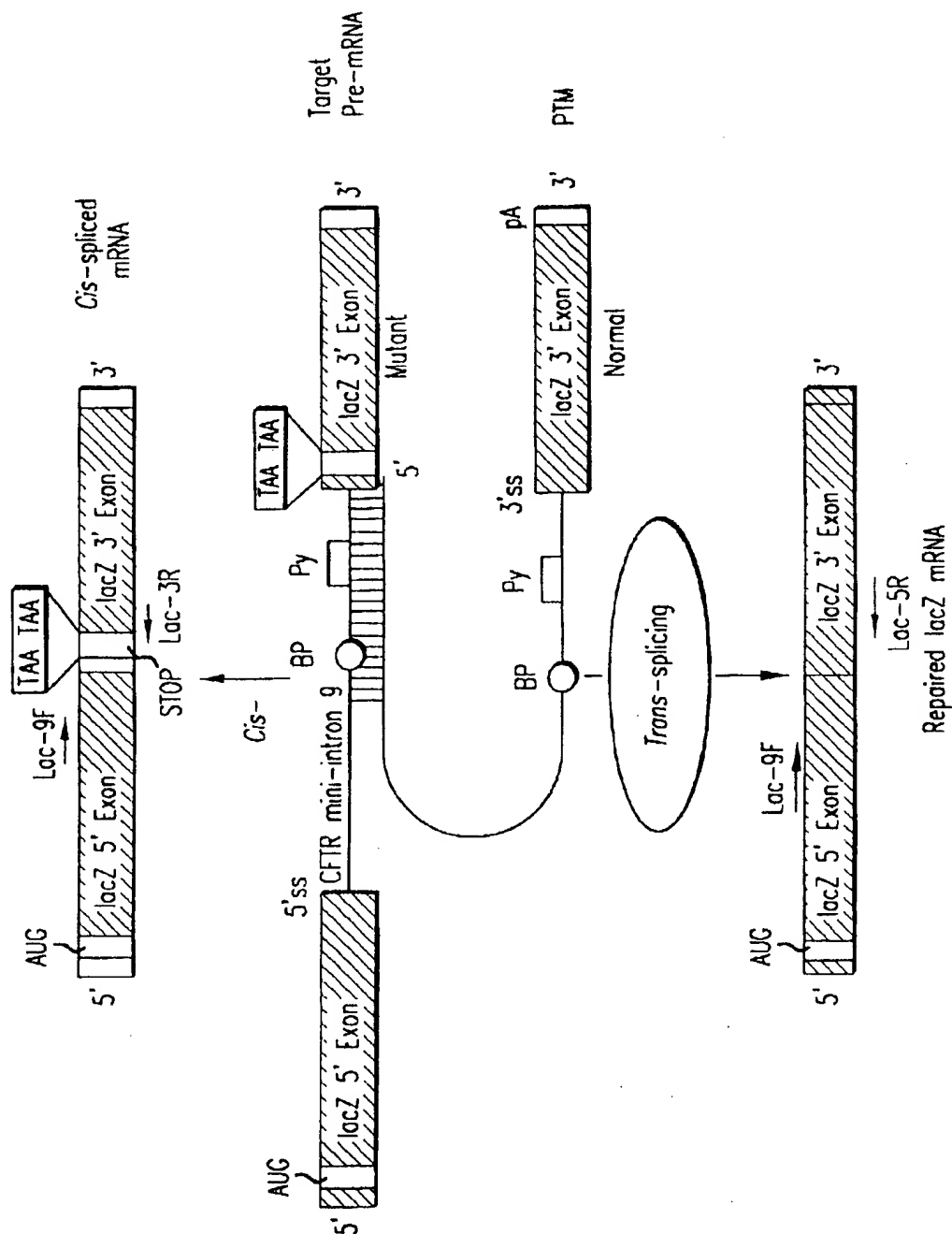


FIG.37C

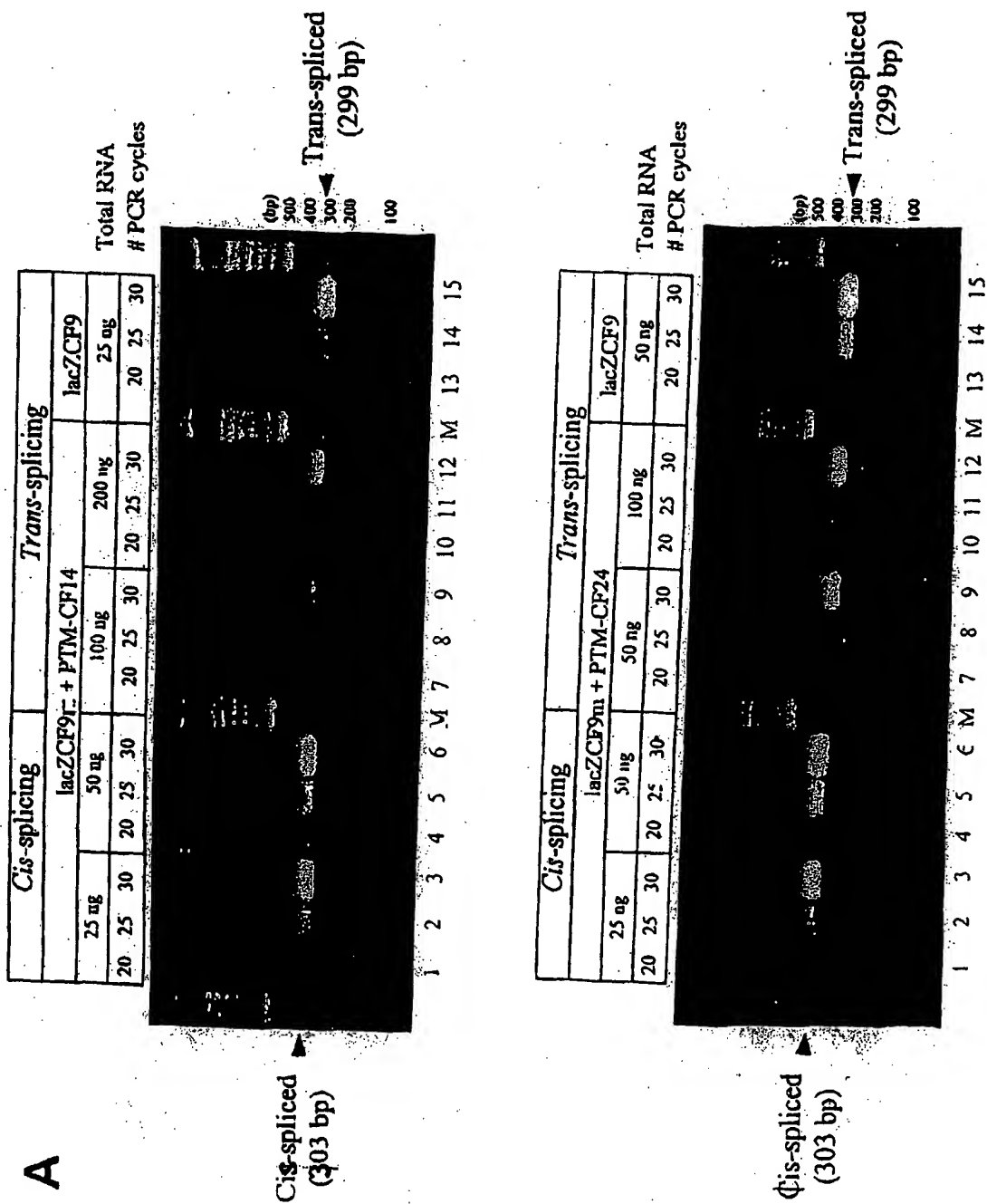


FIG. 38A

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B

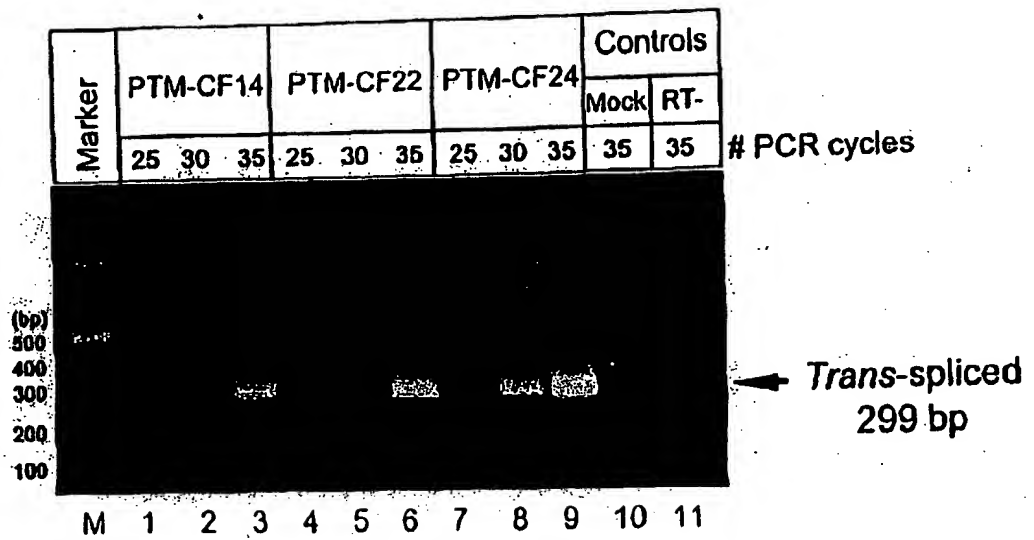


FIG. 38B

204040" 25717550

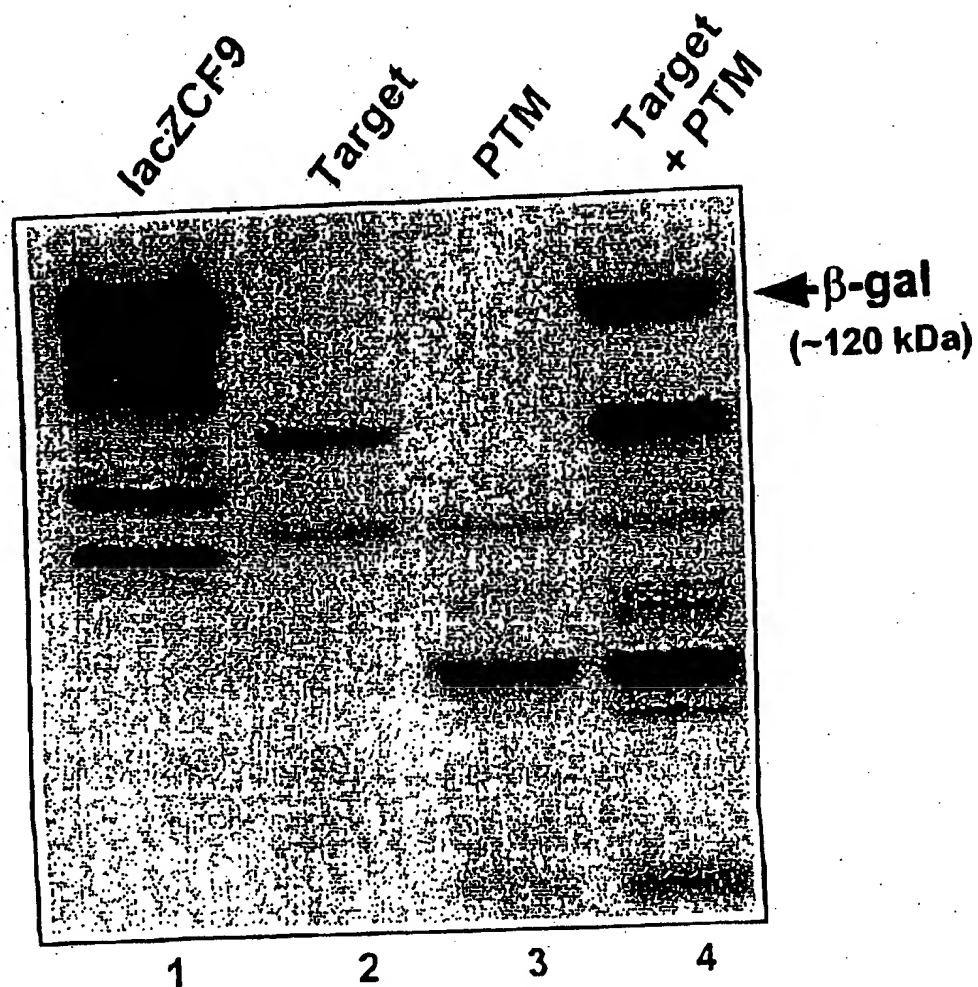


FIG. 39

204040" 25474650

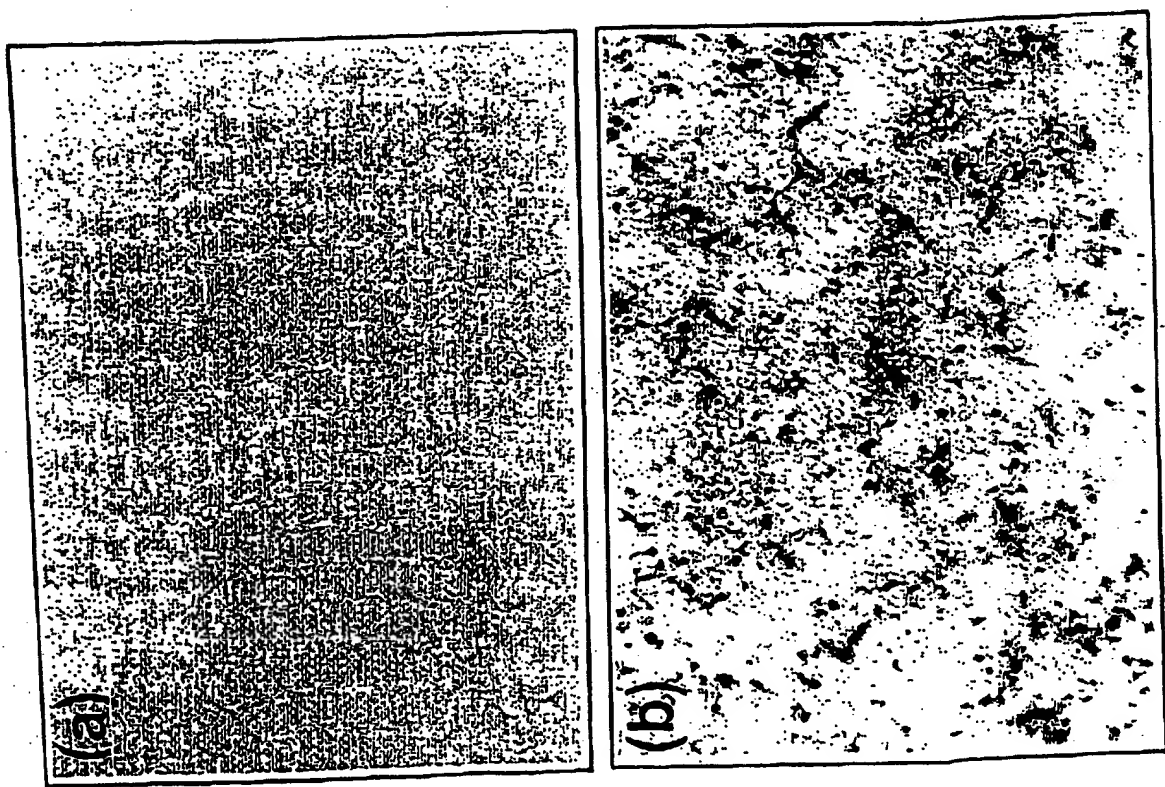


FIG. 40A

A

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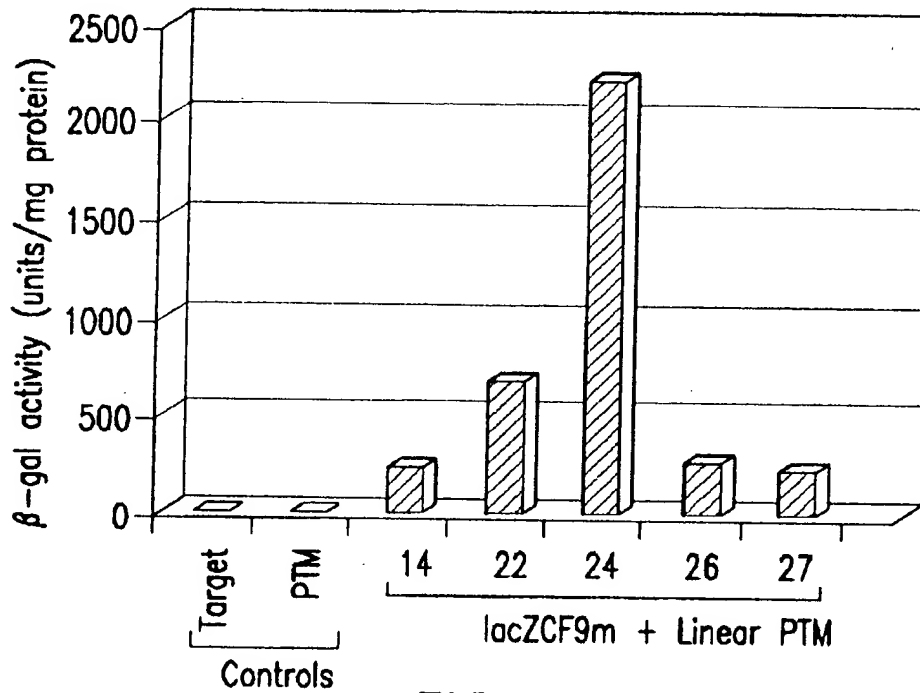


FIG.40B

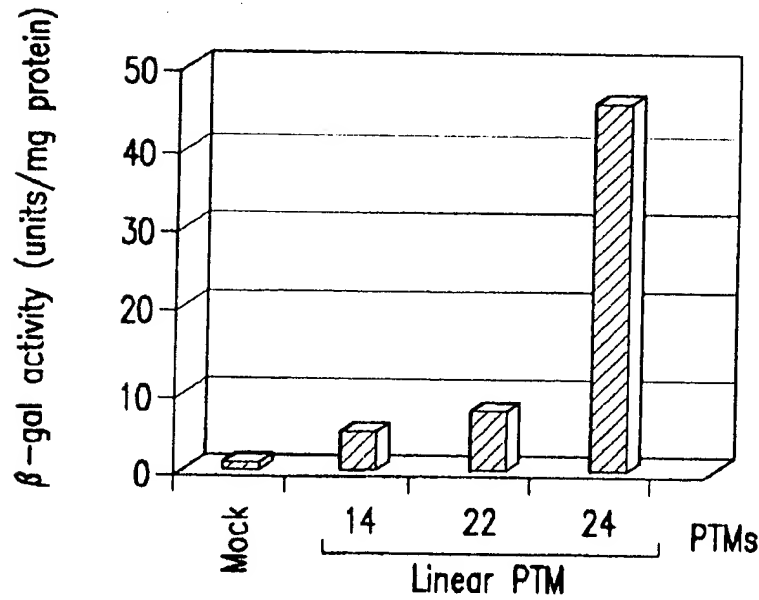


FIG.40C

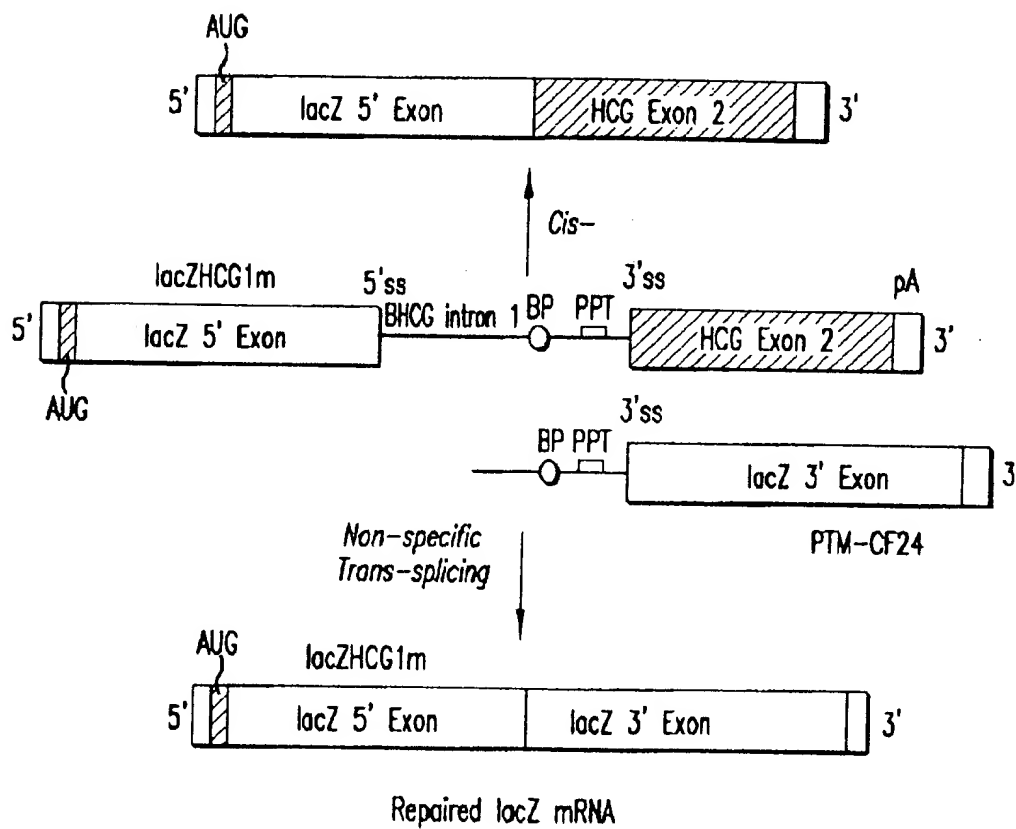


FIG.41A

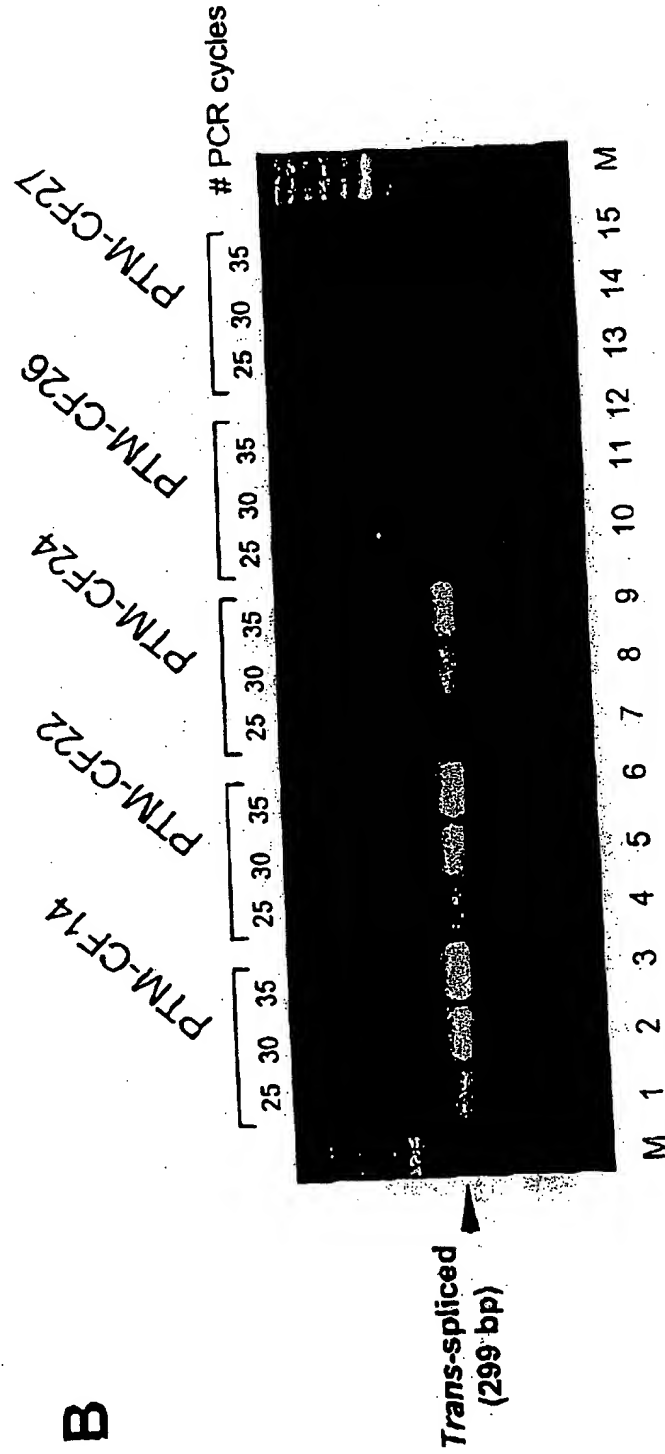


Figure 4B

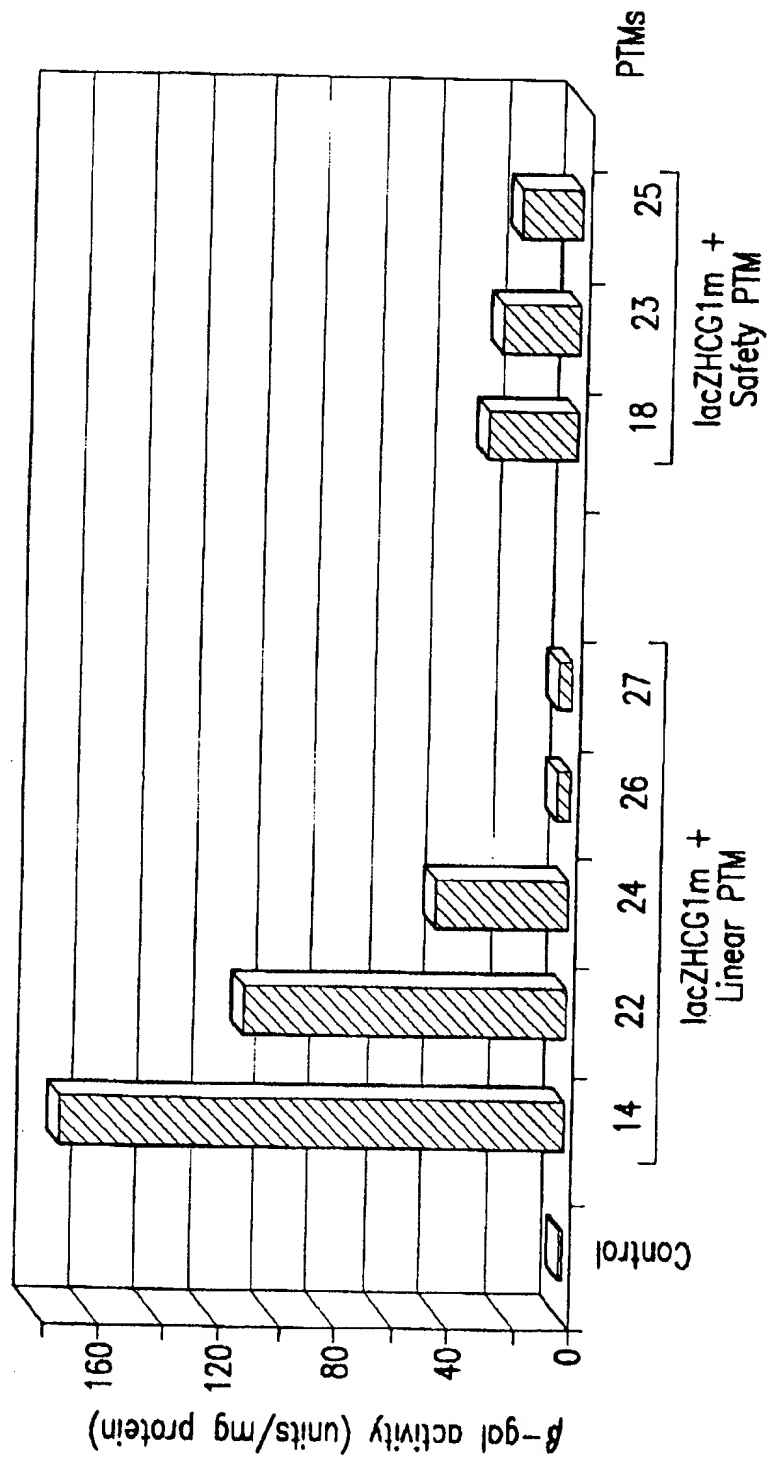


FIG.41C

Exons
1-10

ATGCAGAGGTCGCCTCTGGAAGGCCAGCGTTGTCTCCAACTTTTTTTCAGCTGGACCAGACCAATTTTGAGGAAAG
 GATACAGACAGCGCCTGGAATTGTCAGACATATACCAATCCCTTCTGTTGATTCTGCTGACAATCTATCTGAAAAATT
 CGAAAGAGAATGGGATAGAGAGCTGGCTTCAAAGAAAAATCCTAAACTCATTAAATGCCCTTCGGCGATGTTTTTCTGG
 AGATTTATGTTCTATGGAATCTTTTATATTTAGGGGAAGTCACCAAGCAGTACAGCCTCTCTTACTCGGAAGAATCA
 TAGCTTCTATGACCGGATAACAAGGAGGAACGCTCTATCGCGATTATCTAGGCATAGCCTTATGCCTTCTCTTTAT
 TGTGAGGACACTGCTCTACACCCAGCCATTTTGGCCTTCATCACATGGAATCCAGATGAGAATAGCTATGTTTAGT
 TTGATTTATAAGAAGACTTTAAAGCTGTCAAGCCGTGTTCTAGATAAAATAAGTATTGGACAACCTTGTAGTCTCCTTT
 CCAACAACCTGAACAAATTTGATGAAGGACTTGCATTGGCACATTTCTGTGGATCGCTCCTTTGCAAGTGGCACTCCT
 CATGGGCTAATCTGGGACTTGTACAGGCGTCTGCCCTCTGTGGACTTGGTTTCCTGATAGTCCTTGCCCTTTTTCAG
 GCTGGGCTAGGAGAATGATGATGAAGTACAGAGATCAGAGAGCTGGGAAGATCAGTGAAAGACTTGTGATTACCTCAG
 AAATGATCGAGAACATCCAATCTGTTAAGGCATACTGCTGGGAAGAAGCAATGGAAAAATGATTGAAAACCTAAGACA
 AACAGAACTGAAACTGACTCGGAAGGCAGCCTATGTGAGATACTTCAATAGCTCAGCCTTCTCTCTCAGGGTCTTT
 GTGGTGTTTTTATCTGTGCTTCCCTATGCACTAATCAAAGGAATCATCCTCCGAAAAATATTACCACCATCTCATTCT
 GCATTGTTCTGCGCATGGCGTCACTCGGCAATTTCCCTGGGCTGTACAAACATGGTATGACTCTCTTGGAGCAATAAA
 CAAAATACAGGATTTCTTACAAAAGCAAGAATATAAGACATTGGAATATACTTAACGACTACAGAAGTAGTGATGGAG
 AATGTAACAGCCTTCTGGGAGGAGGATTTGGGGAATTATTGAGAAAGCAAAACAAACAATAACAATAGAAAACTT
 CTAATGGTGATGACAGCCTCTTCTTCAGTAATTTCTCACTTCTTGGTACTCCTGTCTGAAAGATTAATTTCAGAT
 AGAAGAGGACAGTTGTTGGCGTTGCTGGATCCACTGGAGCAGGCAAGACGAGCTTGCTCATGATGATCATGGGCGAG
TTAGAACCAAGTGAAGGCAAGATCAAACATTCCGCGGCATCAGCTTTGCAGCCAATTCAGTTGGATCATGCCCGTA
CCATCAAGGAGAACAATAATCTTCGGGT CAGTTACGACGATACCGCTATCCCTGGTGATTAAAGCCCTGTCAGTTGGA
GGAG

Trans-splicing domain

GTAAGATATCACCGATATGTGTCTAACCTGATTCCGGCCTTCGATACGCTAAGATCCACCGG
TCAAAAAGTTTTACATAATTTCTTACCTCTTCTTGAATTCATGCTTTGATGACGCTTCTGTATCTATATTCATCATTG
GAAACACCAATGATATTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTTCTTCCACTGT
GCTTAATTTTACCCTCTGAATTTCTCCATAATCATATTACAACCTGAACCTCGGAAATAAAACCATCATT
ATTAACCTATTATCAAAATCACGCT

FIG.42

153 bp PTM24 Binding Domain:

Nhe I

153 bp BD underlined

GCTAGC-AAATAATGACGAAGCGCGCCCTCAGGCTCAGGATTCACCTTGCCCTCCAATTATCATCCTAAGCAGAAGTGTATA

TTCATTGTTGTAAGATTCTATTAACTCAATTGATTCAAAATATTTAAATACTTCCTGTTTACCTACTCTGCTATGC

Sac II

AC-CCGCGG

FIG.43A

Trans-splicing domain

AATAATGACGAAGCGCCCTCAGGCTCAGGATTCACCTTGGCCCTCCAATTATCATCCTAAGCAGAAGTGTATATCTTA
TTTGTAAGATTCTATTAACCTCATTGATTCAAATATTTAAATACTTCCTGTTTACCTACTCTGCTATGCACCCGC
GGAACATTATTATAACGTTCCTCGAATACTAAGTGGTACCTCTCTTTTTTTTGATATCCTGCAG

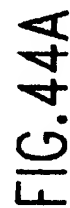
Exons 10-24

ACTTCACTTCTAATGATGATTATGGGAGAACTGGAGCCTTCAGAGGGTAAAATTAAGCACAGTGAAGAATTTCATTCT
 GTTCTCAGTTTTCTGGATTATGCCTGGCACCATTAAAGAAAATATCATCTTTGGTGTTCCTATGATGAATATAGATA
 CAGAAGCGTCATCAAAGCATGCCAACTAGAAGAGGACATCTCCAAGTTTCAGAGAAAGACAATATAGTTCTTGGAGAA
 GGTGGAATCACACTGAGTGGAGGTCAACGAGCAAGAATTTCTTTAGCAAGAGCAGTATACAAAGATGCTGATTGTATT
 TATTAGACTCTCCTTTTGGATACCTAGATGTTTTAACAGAAAAAGAAATATTGAAAGCTGTGTCTGTAACCTGATGGC
 TAACAAAAGTAGGATTTTGGTCACTTCTAAAATGGAACATTTAAAGAAAGCTGACAAAATATTAATTTTGCATGAAGGT
 AGCAGCTATTTTTATGGGACATTTTCAGAACTCCAAAATCTACAGCCAGACTTTAGCTCAAACTCATGGGATGTGATT
 CTTTCGACCAATTTAGTGCAGAAAGAAGAAATCAATCTTAAGTACAGCTTACACCGTTTCTCATTAGAAGGAGATGC
 TCCTGTCTCCTGGACAGAAACAAAAACAATCTTTTAAACAGACTGGAGAGTTTGGGAAAAAGGAAGATTCTATT
 CTCAATCCAATCAACTCTATACGAAAATTTCCATTGTGCAAAAGACTCCCTTACAAATGAATGGCATCGAAGAGGATT
 CTGATGAGCCTTTAGAGAGAAGGCTGTCTTAGTACCAGATTCTGAGCAGGAGAGGGGATACCTCGCATCAGCGT
 GATCAGCACTGGCCCCACGCTTCAGGCACGAAGGAGGAGTCTGTCTGAACCTGATGACACACTCAGTTAACCAAGGT
 CAGAACATTCACCGAAAGACAACAGCATCCACACGAAAAGTGTCACTGGCCCTCAGGCAAACTTGACTGAACCTGGATA
 TATATTCAAGAAGGTTATCTCAAGAACTGGCTTGGAAATAAGTGAAGAAATTAACGAAGAAGACTTAAGGAGTGCTT
 TTTTGATGATATGGAGAGCATACAGCAGTGACTACATGGAACATACCTTCGATATATTACTGTCCACAAGAGCTTA
 ATTTTGTGCTAATTTGGTGCTTAGTAATTTTCTGGCAGAGGTGGCTGCTTCTTTGGTTGTCTGTGCTCCTTGGAA
 AACTCCTCTTCAAGACAAGGGAATAGTACTCATAGTAGAAATAACAGCTATGCAGTGATTATCACCAGCACCAGTTT
 GTATTATGTGTTTTACATTACGTGGGAGTAGCCGACACTTTGCTTGCTATGGGATTCTTCAGAGGTCTACCAGTGGT
 CATACTCTAATCAGAGTGTGAAAATTTTACACCACAAAATGTTACATTCTGTTCTTCAAGCACCTATGTCAACCTCA
 ACACGTGAAAGCAGGTGGGATTCTTAATAGATTCTCCAAGATATAGCAATTTTGGATGACCTTCTGCCCTCTACCAT
 ATTTGACTTCATCCAGTTGTTATTAATTTGATTGGAGCTATAGCAGTTGTGCGAGTTTTACAACCTACATCTTTGTT
 GCAACAGTGCCAGTGATAGTGGCTTTTATTATGTTGAGAGCATATTTCTCCAACCTCACAGCAACTCAAACAACCTGG
 AATCTGAAGGCAGGAGTCCAATTTTCACTCATCTTGTACAAGCTTAAAGGACTATGGACACTTCGTGCCCTTCGGACC
 GCAGCCTTACTTTGAAACTCTGTTCACAAAGCTCTGAATTTACATACTGCCAACTGGTCTTGTAACCTGTCAACACTG
 CGCTGGTTCCAAATGAGAATAGAAATGATTTTGTGATCTTCTTCATTGCTGTTACCTTCATTTCCATTTTAAACAAG
 GAGAAGGAGAAGGAAGAGTTGGTATTATCTGACTTAGCCATGAATATCATGAGTACATTGCAGTGGCTGTAAACTC
 CAGCATAGATGTGGATAGCTTGATGGATCTGTGAGCCGAGTCTTTAAGTTCAATTGACATGCCAACAGAAGGTAAACCT
 ACCAAGTCAACCAACCATAAGAATGGCCAACCTCTCGAAAGTTATGATTATTGAGAATTCACAGGTGAAGAAAGATG
 ACATCTGGCCCTCAGGGGGCCAAATGACTGTCAAAGATCTCAGCAAAATACACAGAAGGTGGAATGCCATATTAGA
 GAACATTTCTTCTCAATAAGTCTGGCCAGAGGTGGGCTCTTGGGAAGAACTGGATCAGGGAAGAGTACTTTGTTA
 TCAGCTTTTTGAGACTACTGAACACTGAAGGAGAAATCCAGATCGATGGTGTGCTTGGGATTCAATAACTTTGCAAC
 AGTGGAGGAAAGCCTTTGGAGTGATACACAGAAAGTATTTATTTTTCTGGAACATTAGAAAAAACTGGATCCCTA
 TGAACAGTGGAGTGATCAAGAAATATGGAAGTTGCAGATGAGGTGGGCTCAGATCTGTGATAGAACAGTTTCTGGG
 AAGCTTGACTTTGTCTTGTGGATGGGGCTGTGCTCTAAGCCATGGCCACAAGCAGTTGATGTGCTTGGCTAGATCTG
 TTCTCAGTAAGCGGAAGATCTTGCTGCTTGATGAACCCAGTGCCTATTGGATCCAGTAACATACCAATAATTACAAG
 AACTCTAAAACAAGCATTTGCTGATTGCACAGTAATTTCTGTGAACACAGGATAGAAGCAATGCTGGAATGCCAACAA
 TTTTTGCTCATAGAAGAGAACAAGTGGCGGAGTACGATTCATCCAGAACTGCTGAACGAGAGGAGCCTCTTCCGGC
 AAGCCATCAGCCCTCCGACAGGGTGAAGCTCTTTCCCAACCGAACTCAAGCAAGTGAAGCTTAAGCCCCAGATTGC

Histidine log Stop

TGCTCTGAAAGAGGAGACAGAAGAAGAGGTGCAAGATACAAGGCTTCATCATCATCATCATTAG

FIG.43B



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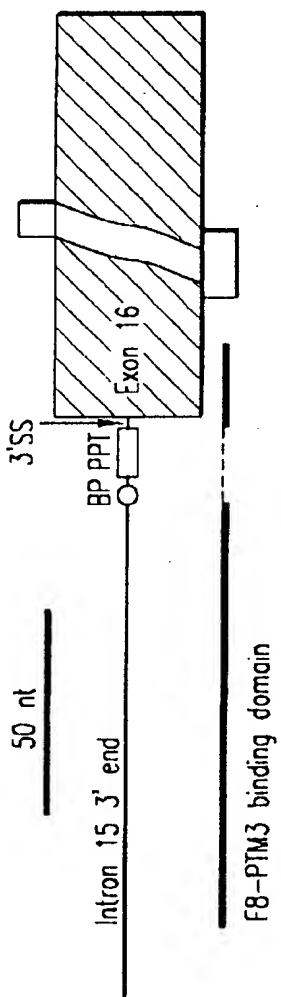


FIG. 44B

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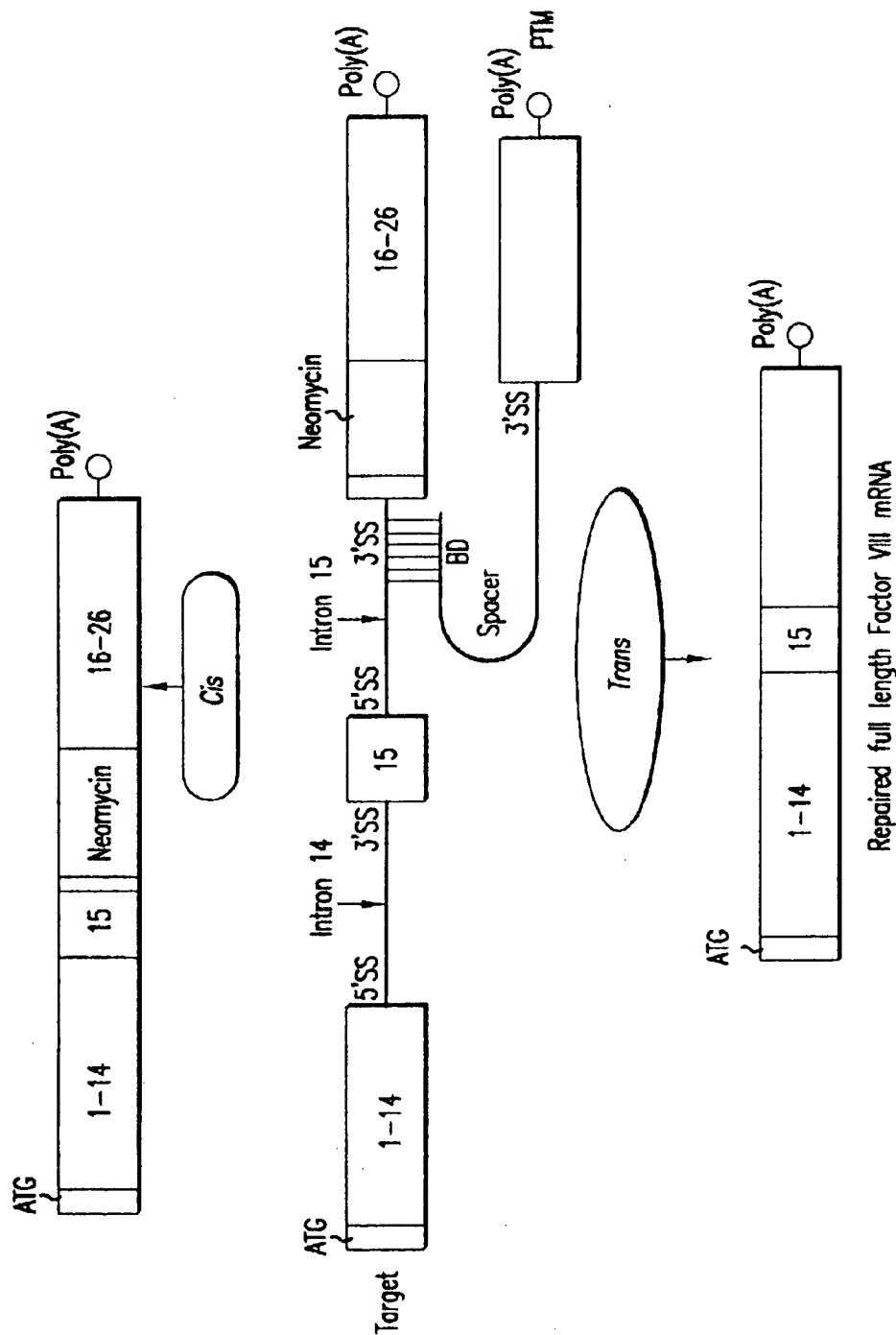
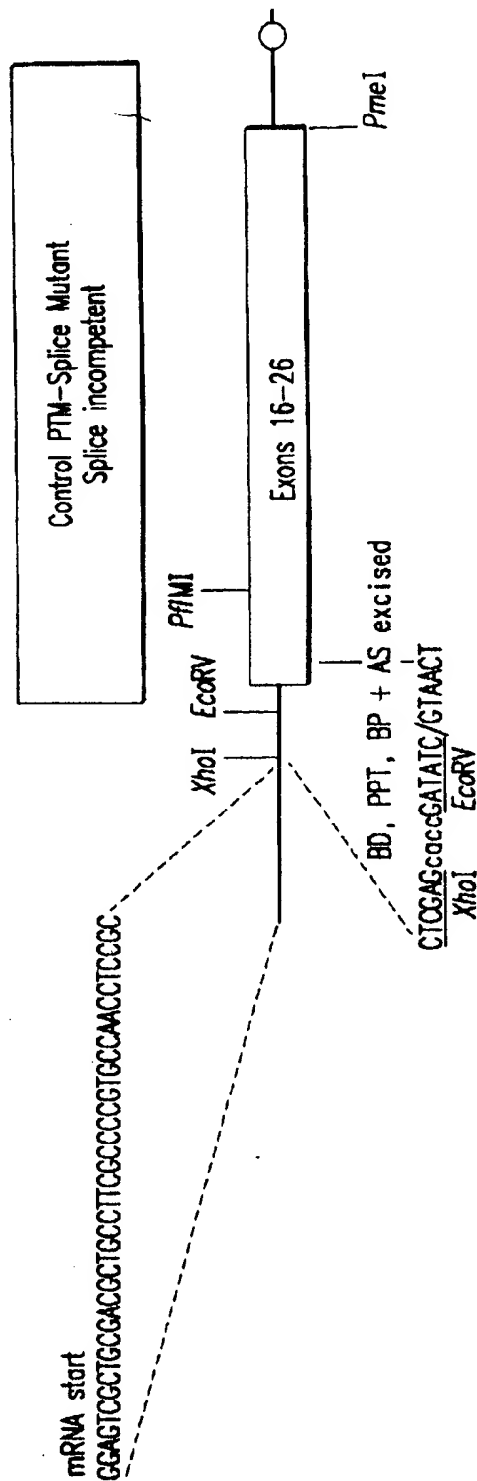


FIG.44D

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Method:
Excise TSD and part of exon 16 with
XhoI and PflMI and ligate in a PCR product that:
1) eliminates the TSD and splice acceptor site
2) inserts EcoRV adjacent to exon 16
3) restores the coding for exon 16

FIG.45

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Repair of Factor VIII
Preliminary results from one experiment

FVIII activity in Exon 16 FVII-KO mice
after IV PTM-FVII intraportal infusion
(100 μ gDNA)(n=3)

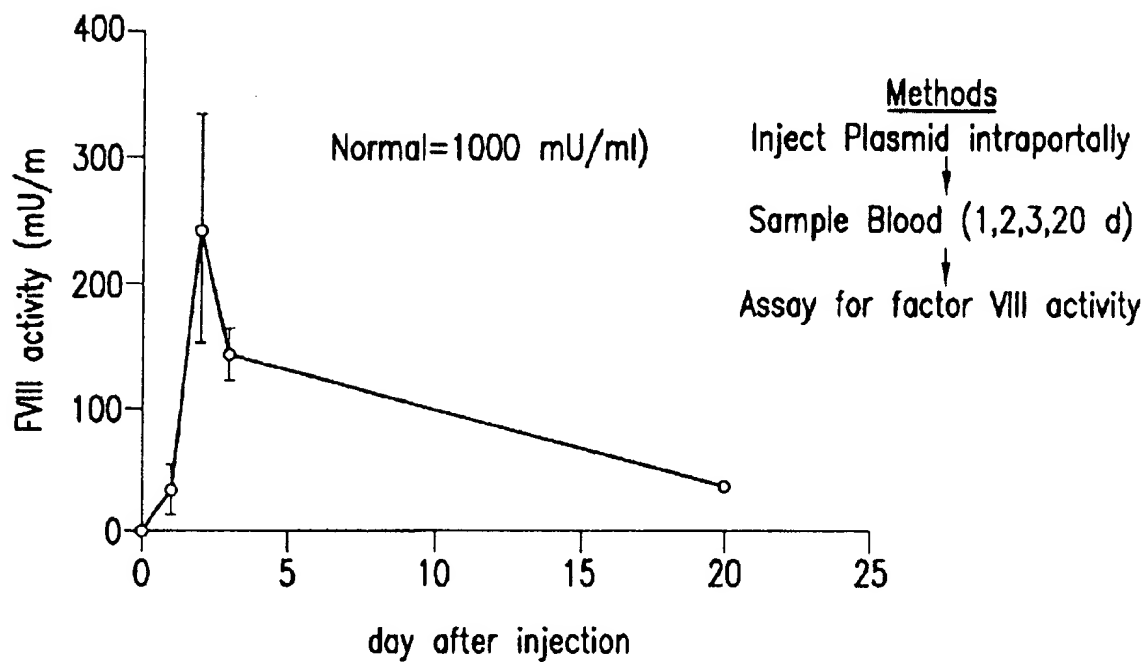


FIG.46

201040" 264460

Detailed structure of a mouse factor VIII PTM containing normal sequences for exons 16-26 and a C-terminal FLAG tag. BGH=bovine growth hormone 3' UTR; Binding domain= 125 bp.

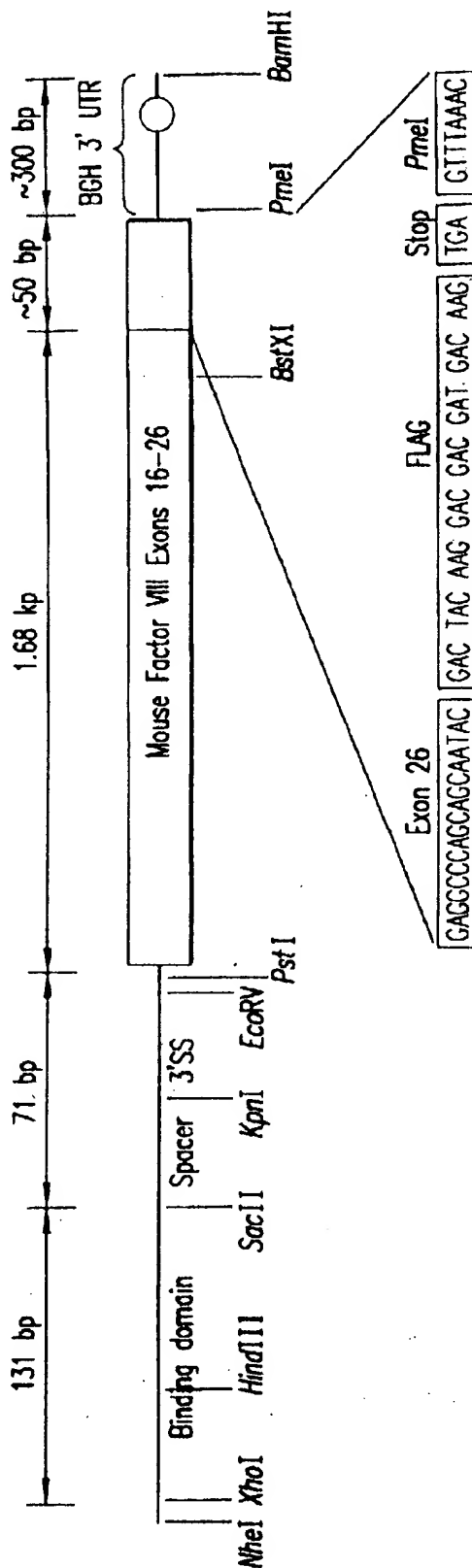


FIG.47A

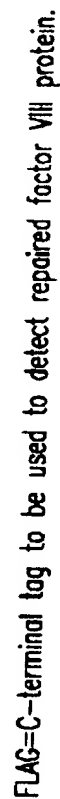


FIG. 47B

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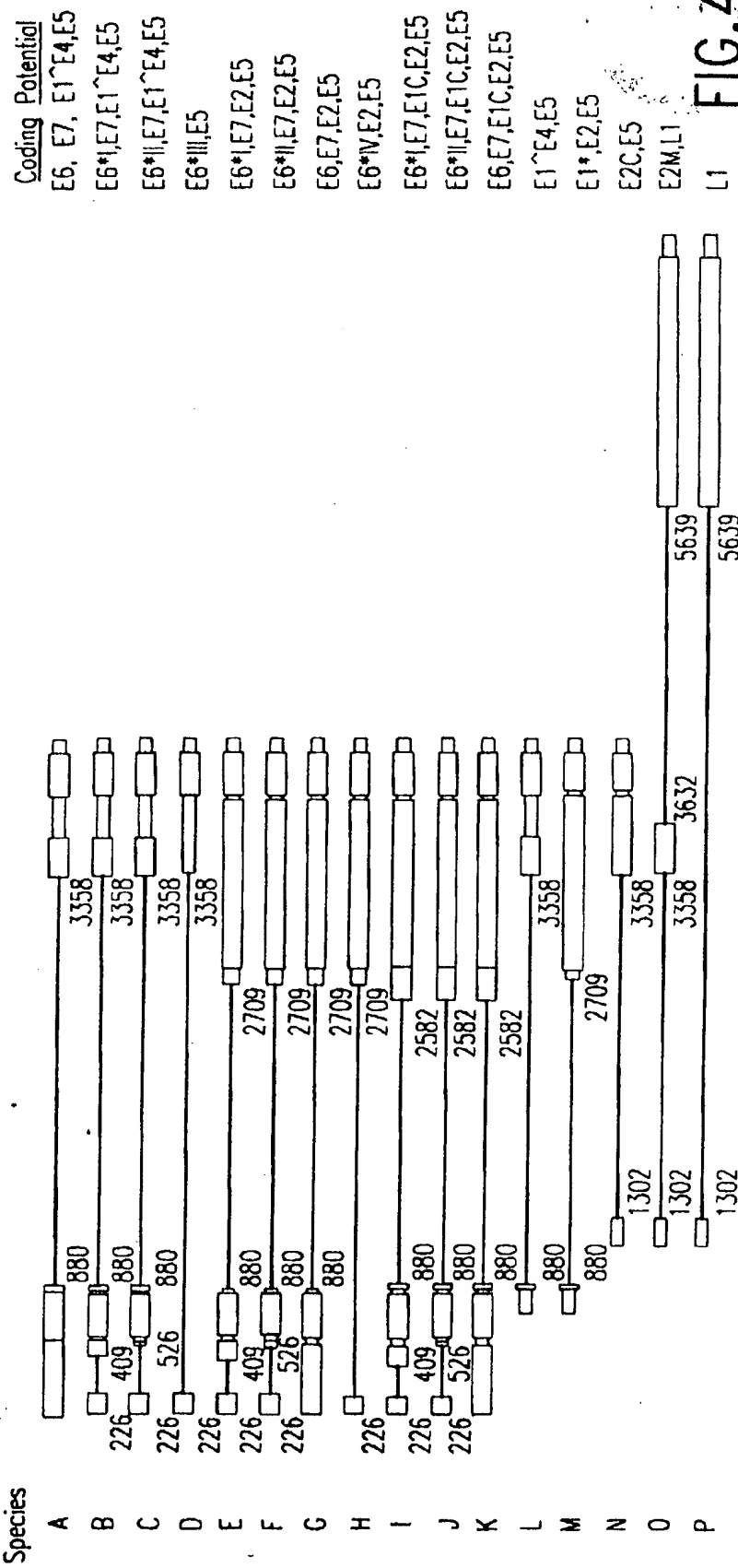
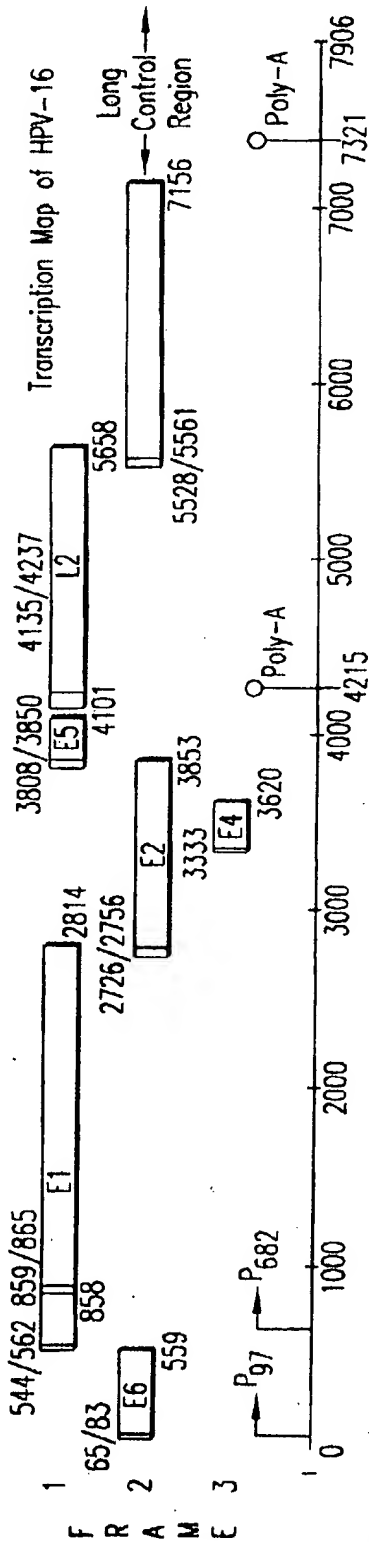
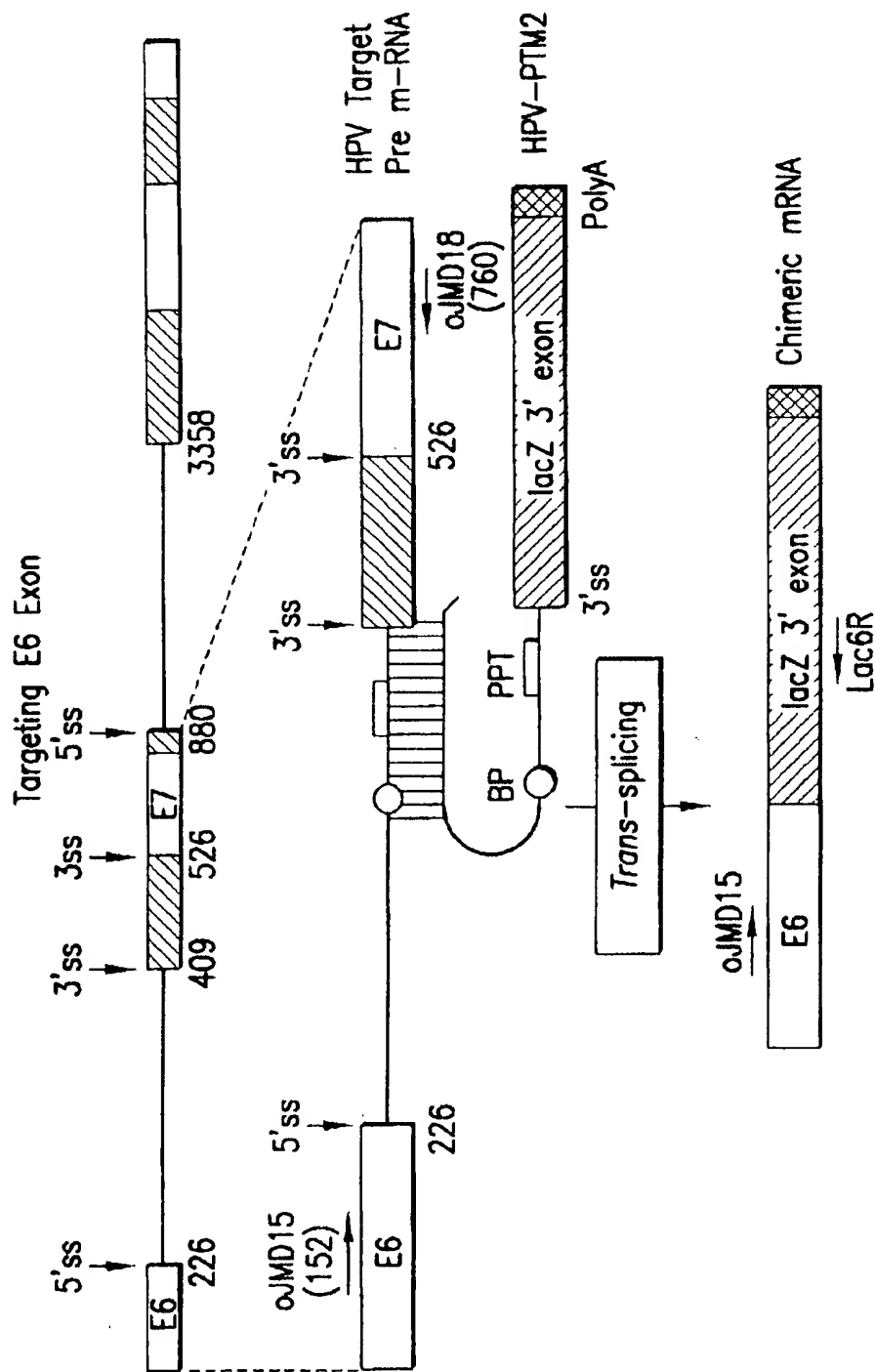


FIG. 48

SMART Strategy to Disrupt the Expression of Human Papillomavirus Type 16



SMART Strategy by 3' Exon Replacement: Schematic diagram of HPV-PTM2 binding to the 3' splice site of the HPV type 16 target pre-mRNA

FIG.49

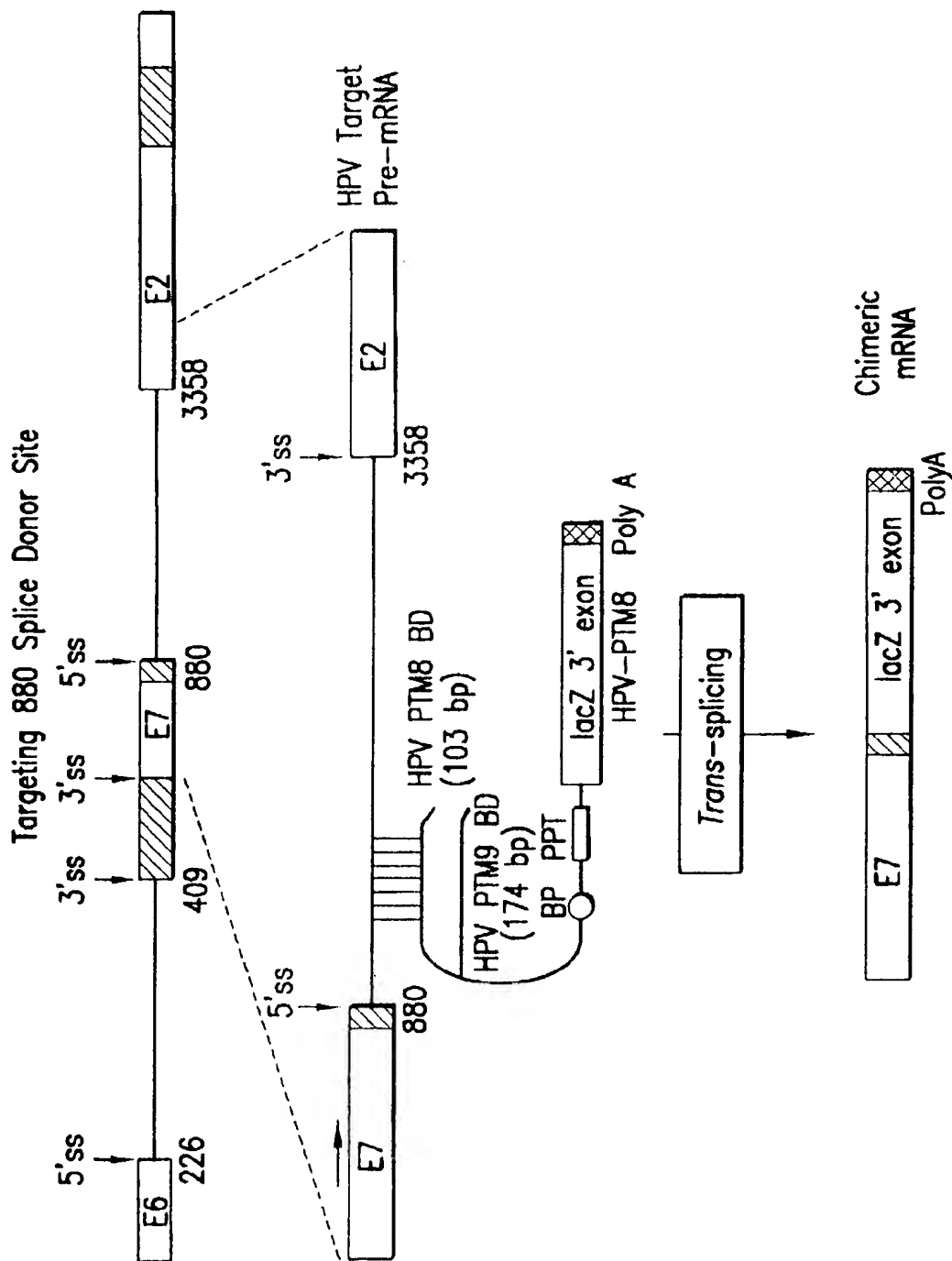


FIG.50

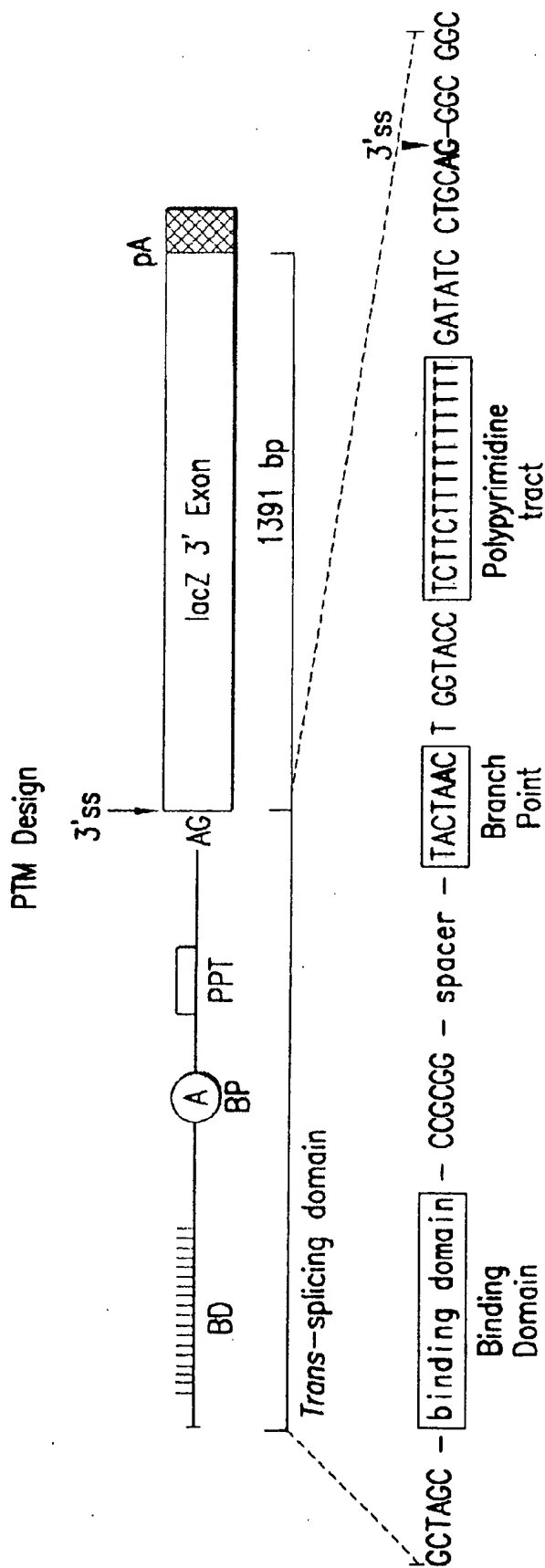


FIG.51

The diagram illustrates the HPV-PTM1 construct and its trans-splicing domain. The construct is 1391 bp and contains a lacZ 3' Exon. The trans-splicing domain is shown with its N and C termini, including the binding domain, spacer, and poly-pyrimidine tract (PPT). The construct is targeted to the 3' splice site (3'ss) at position 409.

HPV-PTM1 construct:

- 3'ss (targeted to 409)
- BD (Binding Domain)
- BP (Branch Point)
- PPT (Poly-pyrimidine Tract)
- lacZ 3' Exon
- 1391 bp

Trans-splicing domain:

- N (N-terminus)
- BD (Binding Domain)
- S (Spacer)
- BP (Branch Point)
- PPT (Poly-pyrimidine Tract)
- C (C-terminus)

Sequence details:

- BD: GCTAGC-binding domain
- S: CCGCGG-spacer
- BP: TACTAC
- PPT: TCTTCTTTT
- C: GGTACC

Binding domain sequence: CAGTTAAATAC ACCTAAATTAA CAAATCACAC AACGCTTTGT TGTATTGCTG
TTCCTAATGTT GTCCCATACA CACTATAACA

FIG. 52A

HPV-PTM2 with 149 bp binding domain targeted to 3' ss at 409:

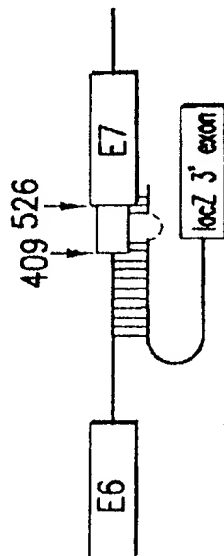
Binding domain sequence: CAGTTAATAC ACCTAATTAA CAAATCACAC AACGCTTTGT TCTATTGCTG
TTC7AATGT GTTCCATACA CACTATAACA ATAATGTCTA TACTCACTAA
TTTTAGAA7A AACCTTTAAA CATT7ATCAC ATACAGCATA TCGATTCCCC

FIG. 52B

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Binding Domains of HPV-PTM3 and 4

HPV-PTM3 Binding domain (covers both 3'ss at 409 and 526; has 53 bp bubble)
 GATGATCTGCAACAAGACATACATCGACCGTCCA(53 nt bubble)CTTCAGGACACAGTGGCTTTTGAC
 AGTTAATACACCTAATTAAACAATCACACAACGGTTTGTGTTATTCAGTTCCTAATGTTGTTCCATACACACTA
 TAACAAT



HPV-PTM4 Binding domain (covers both 3'ss at 409 and 526; has 76 bp bubble)

GATGATCTGCAACAAGAC(76 nt bubble)GACACAGTGGCTTTTGACAGTTAATACACCTAATTACAAATC
 ACACAACGGTTTGTGTTATTCAGTTCCTAATGTTGTTCCATACACACTATAACAAT

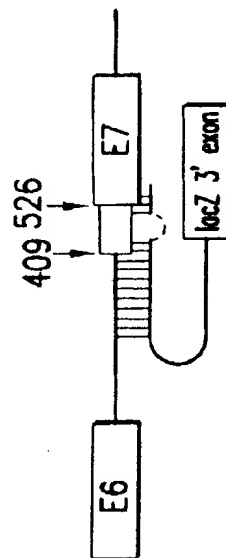
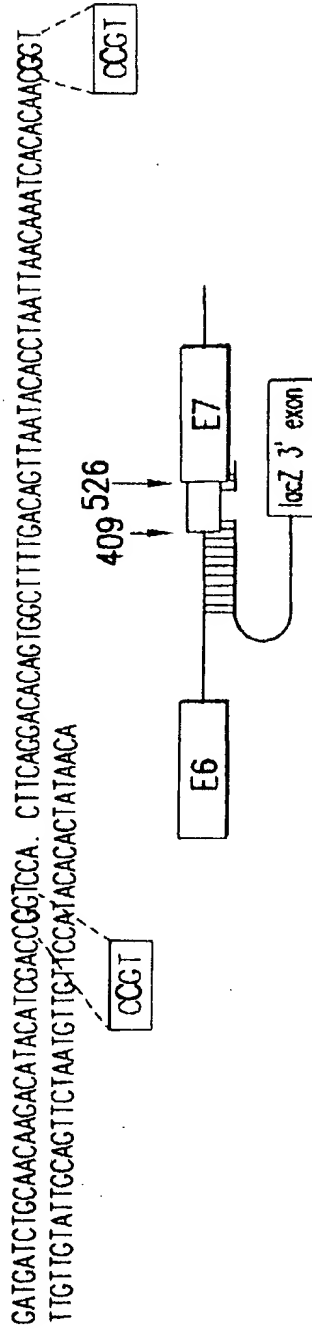


FIG.53

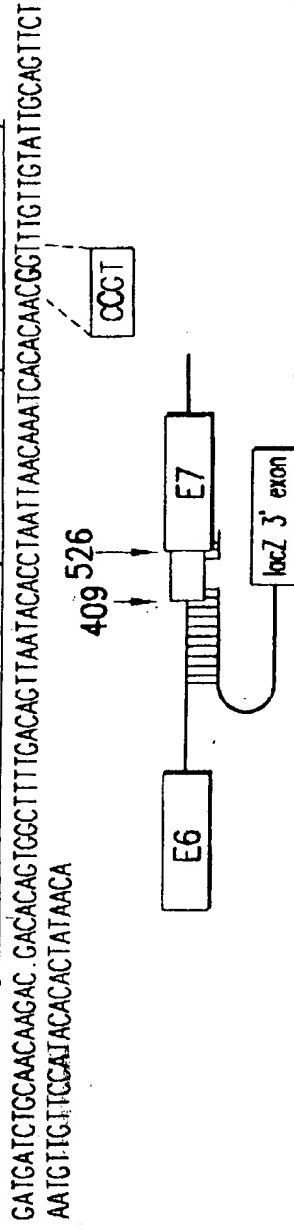
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HPV-PTM5 and 6

HPV-PTM5, Binding domain (140 nt, has 53 nt bubble, covers 3'ss at position 409 and 526)

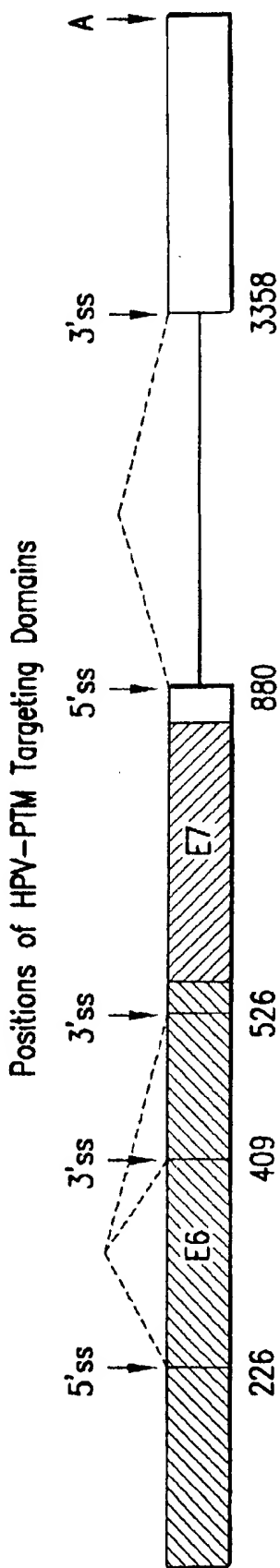


HPV-PTM6, Binding domain (117 nt, has 76 nt bubble, covers 3'ss at position 409 and 526)



Note: Nucleotides in bold are modified to prevent PTMs cryptic splicing

FIG.54

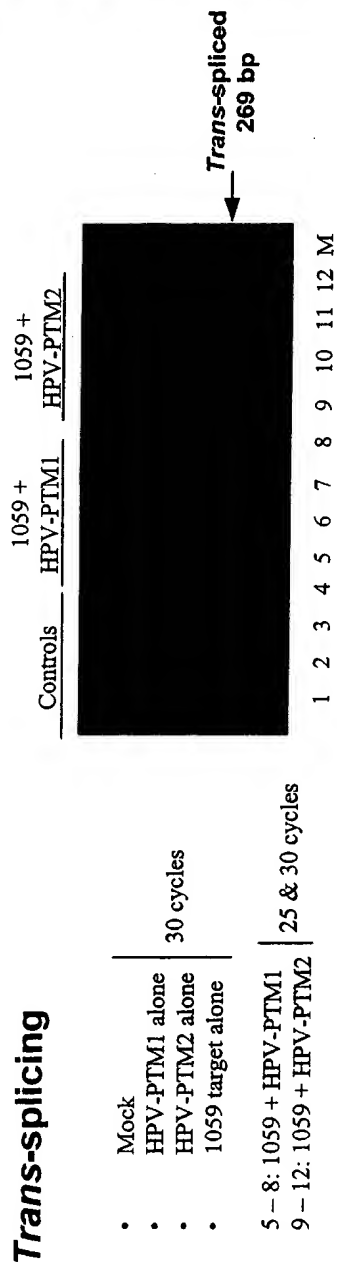
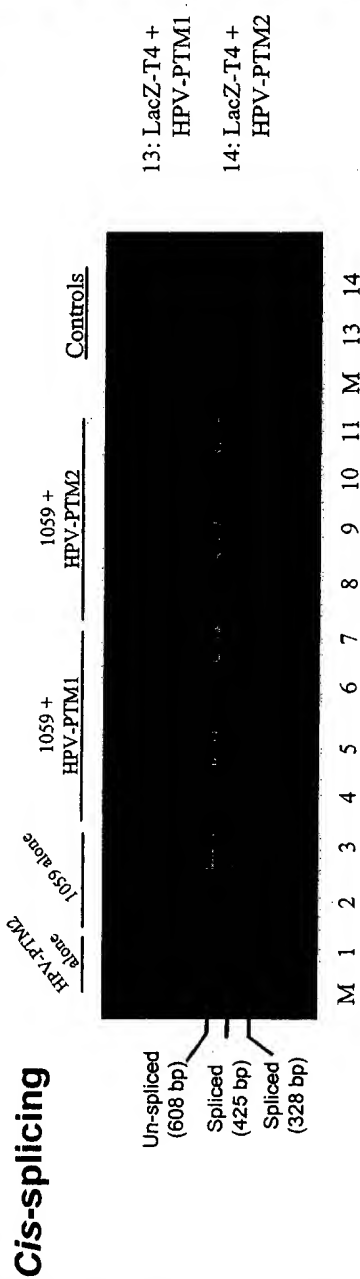


PTM	Binding Domain	
	Region	Size (nt)
HPV-PTM1	A	80
HPV-PTM2	A	149
HPV-PTM5	A+B	140
HPV-PTM6	A+B	117
HPV-PTM8	C	104
HPV-PTM9	C	174

FIG.55

80 2 91

Trans-splicing Efficiency of HPV-PTMs in 293T Cells



RT-PCR Analysis of total RNA

INIRON

FIG. 56

Trans-splicing between target pre-mRNA and
PTM is accurate (293T cells)

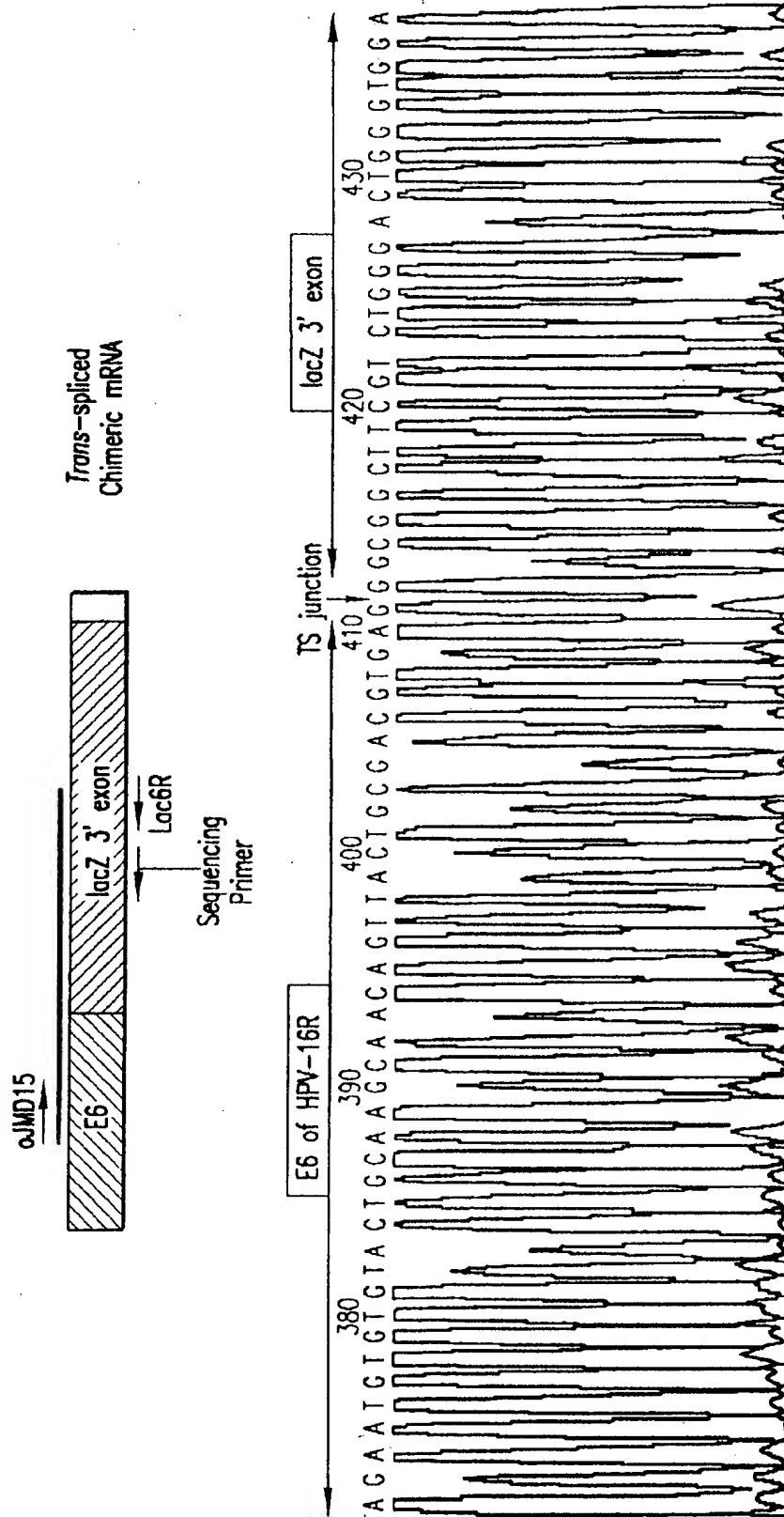
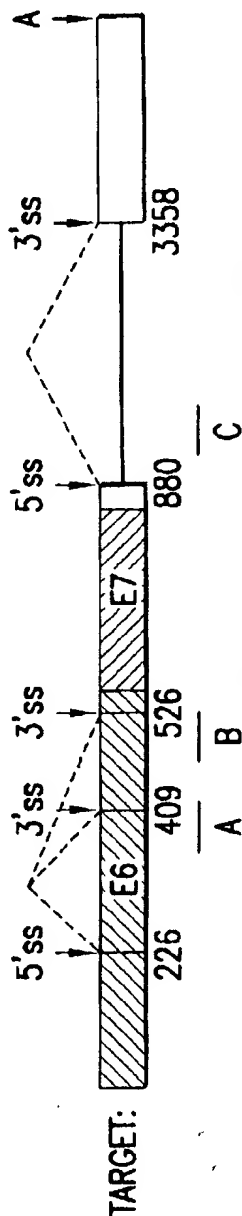


FIG.57

Trans-splicing in 293 Cells (Co-transfections)



PTM	Binding Domain		% trans-spliced	
	Region	Size (nt)	226 sd	880 sd
HPV-PTM1	A	80	69	0.6
HPV-PTM2	A	149	45	0.9
HPV-PTM5	A+B	140	55	0.8
HPV-PTM5ΔBP/PPT	A+B	140	0.5	0.2
HPV-PTM6	A+B	117	59	1
HPV-PTM8	C	104	7	37
HPV-PTM9	C	174	14	22
CF-PTM27	CF intron	411	0	0

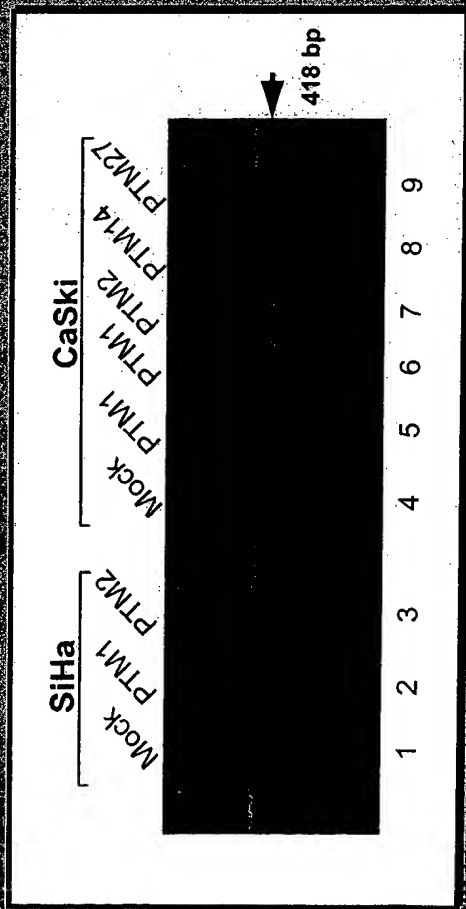
Quantification of Trans-splicing efficiency using real-time QRT-PCR

FIG.58

82 8 91

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Trans-splicing into Endogenous HPV Pre-mRNA Target in SiHa & CaSki Cells



SiHa: Single copy
CaSki: ~400-500 copy/cell



RT-PCR Analysis of total RNA

RT-PCR Conditions

- Total RNA: 400 ng/rxn
- Primer's: oJMD15 + Lac16R
- # Cycles : 35
- Expected product : 418 bp

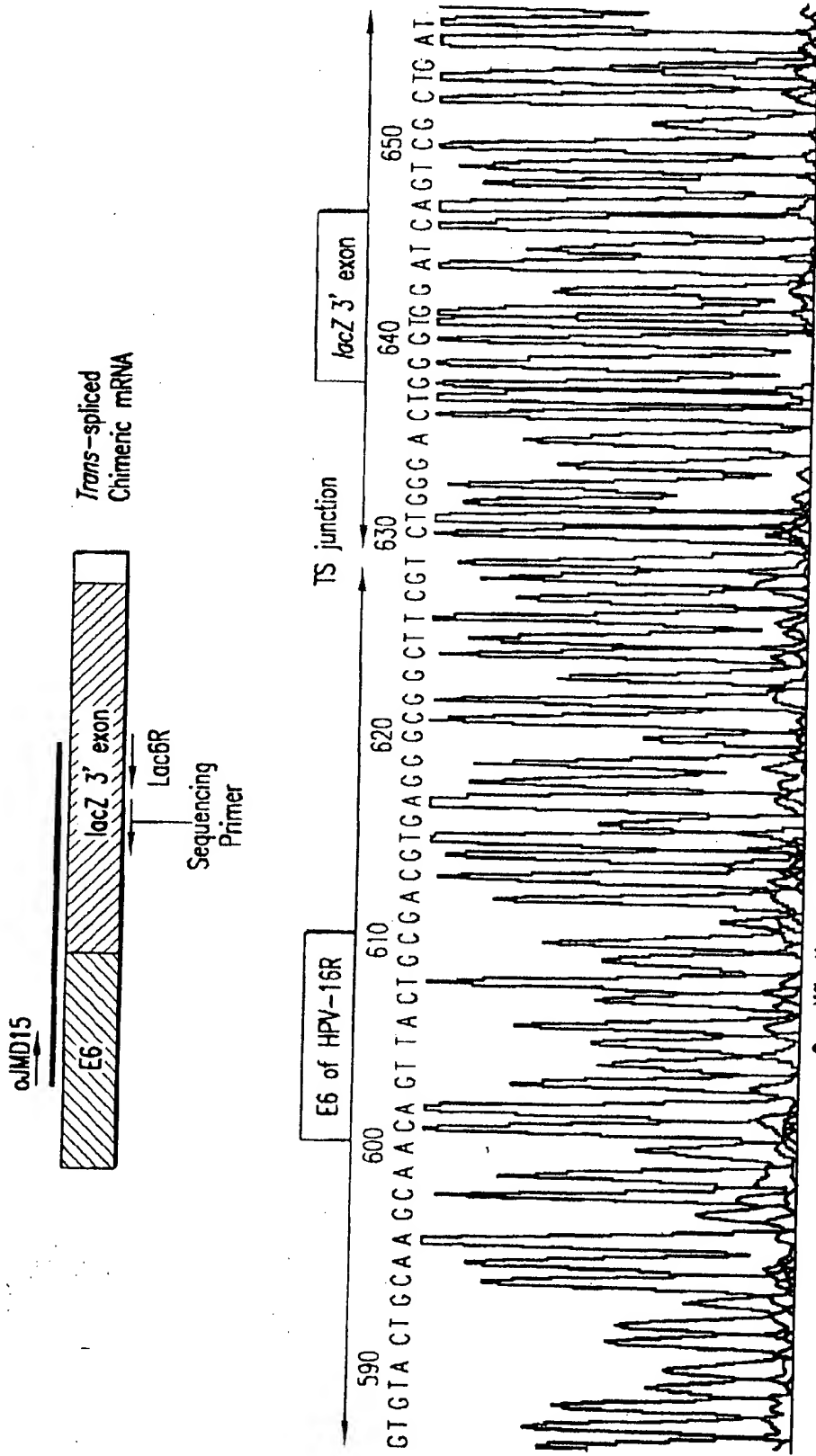
Details

- PTM1, PTM2 : HPV targeted, specific
- PTM14 : CF targeted, non-specific, has 23 bp BD
- PTM14 : CF targeted, non-specific, has 411 bp BD

INTRONN

FIG. 59

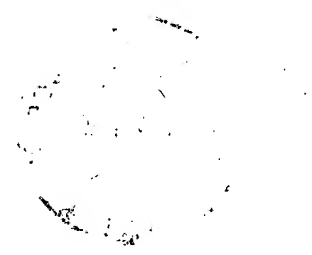
Accurate Trans-splicing of HPV-PTM1
in Si Ha Cells (Endogenous target pre-mRNA)



Quantification of trans-splicing efficiency using real-time QRT-PCR

FIG.60

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Trans-splicing in SiHa Transfections
(Endogenous target)

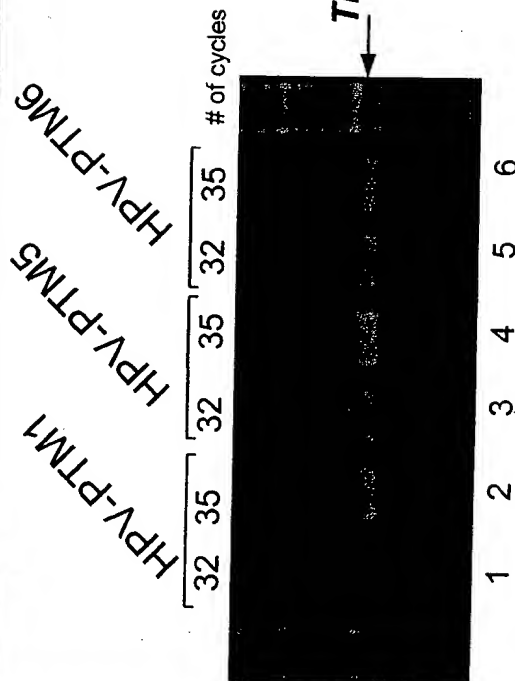
<u>PTM</u>	<u>% trans-spliced</u>
pcDNA3.1	0
HPV-PTM1	0.16
HPV-PTM5	0.12
HPV-PTM6	0.11
CF-PTM27	0

Quantification of *trans*-splicing efficiency using real-time QRT-PCR

FIG.61

20041452 040400

Trans-splicing Efficiency of HPV-PTM1, 5, & 6 in SiHa Cells



• SiHa cells transfected with 1.5 µg plasmid DNA, LipoPlus
• RNA isolated after 48 hr

• Total RNA: 500 ng/Rxn
• Primers: oJMD15 + Lac16R
• Expected product: 418 bp

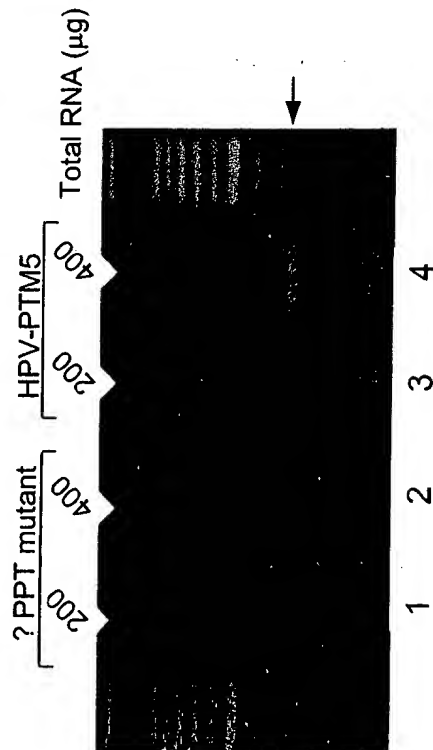
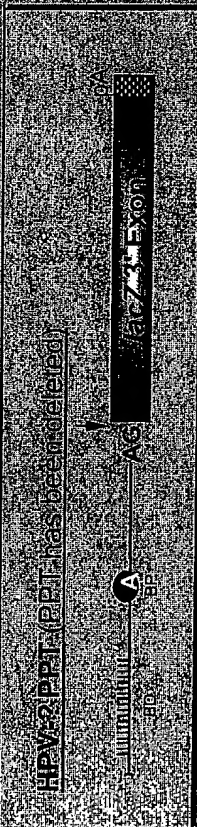
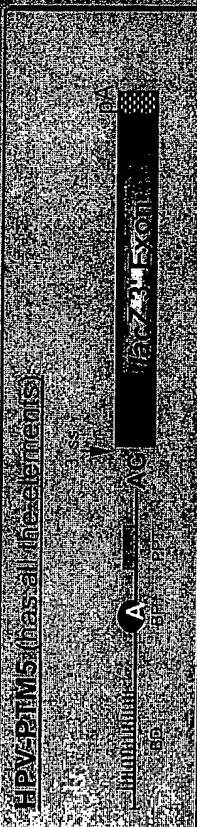
1, 3, 5, : 32 cycles
2, 4, 6, : 35

INTRON

FIG. 62

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Deletion of polypyrimidine tract abolishes *trans*-splicing

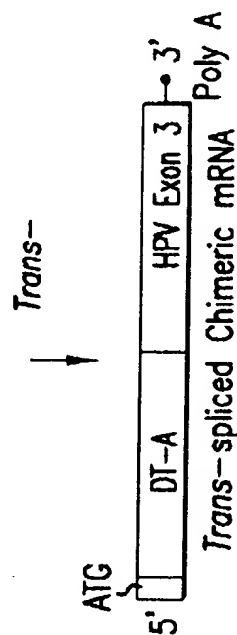
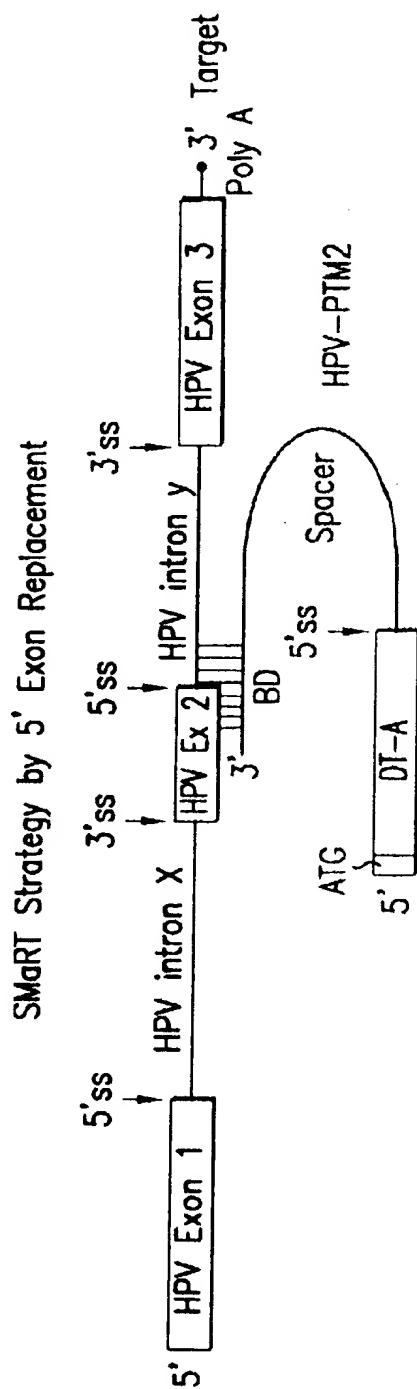


Methods:

- SiHa cells transfected with 1.5 µg of plasmid DNA
 - Total RNA isolated after 48 hr and analyzed by RT-PCR (30 cycles)
- Primers: oJMD15+Lac6R
Expected product: 269 bp
- Lanes 1 & 2: RNA from cells transfected with HPV-? PPT (mutant); No trans-splicing detected
- Lanes 3 & 4: RNA from cells transfected with HPV-PTM5 plasmid; trans-splicing Detected (269 bp product)

INTRONN

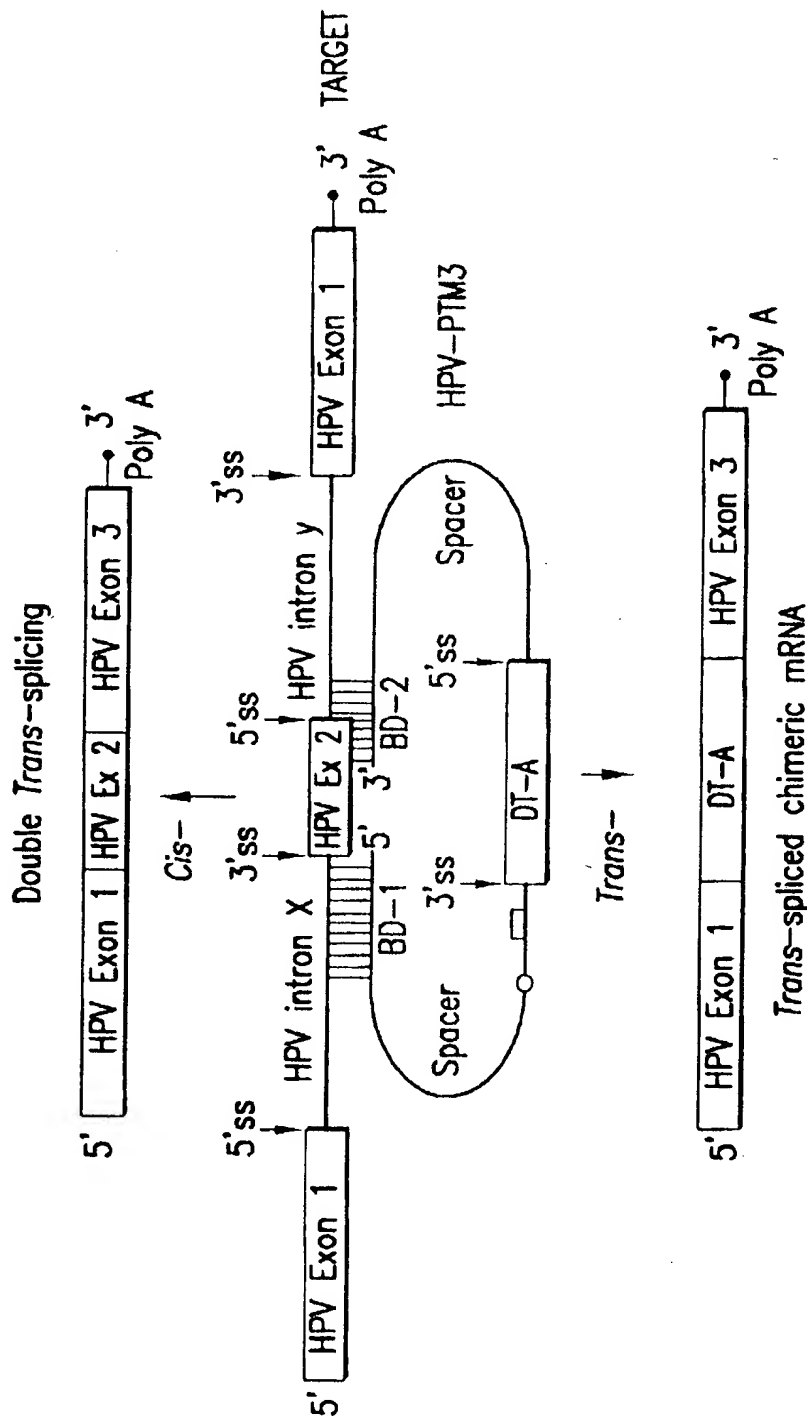
FIG. 63



Schematic diagram of a PTM binding to the 5' splice site of the HPV mini-gene target

FIG.64

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Schematic diagram of a double *Trans*-splicing PTM binding to the 3' and 5' splice sites of the HPV mini-gene target

FIG.65

SMaRT Strategy by 3' Exon Replacement: Schematic diagram of a PTM binding to the 3' splice site of the HPV mini-gene target

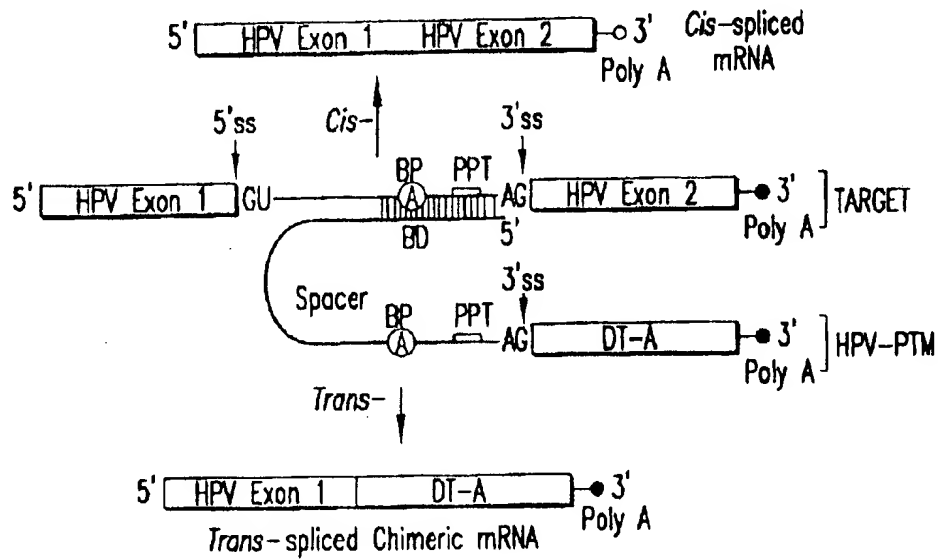


FIG.66A

SMaRT Strategy by 5' Exon Replacement: Schematic diagram of a PTM binding to the 5' splice site of the HPV mini-gene target

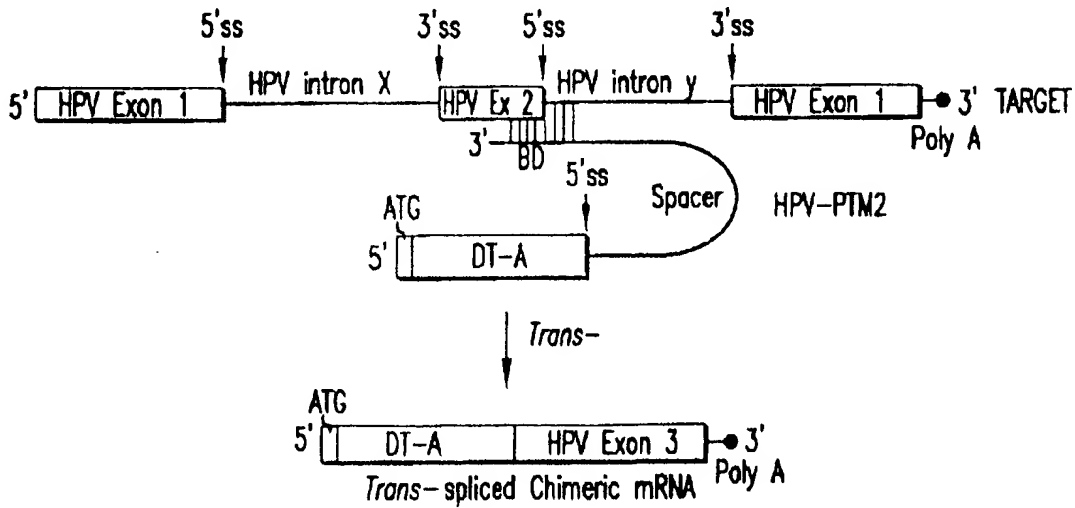


FIG.66B

HPV-PTM3 (For Internal exon replacement)

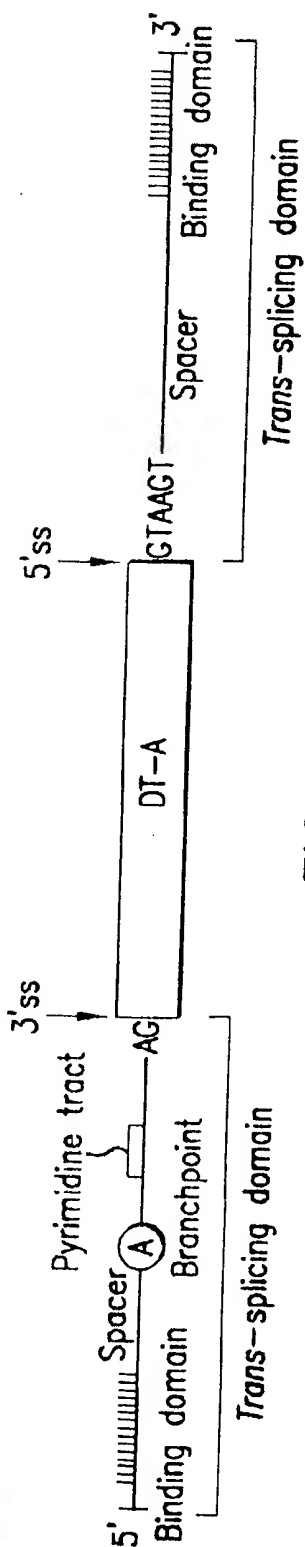


FIG.67

91 8 91